A DNA double-strand break repair defect in

Fanconi anemia fibroblasts

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Running Title: DNA double-strand break repair defect in FA cells
SUMMARY

Fanconi anemia (FA) is a heterogeneous autosomal recessive disease characterized by congenital abnormalities, pancytopenia, and an increased incidence of cancer. Cells cultured from FA patients display elevated spontaneous chromosomal breaks and deletions, and are hypersensitive to bifunctional crosslinking agents. Thus it has been hypothesized that FA is a DNA repair disorder. We analyzed plasmid end-joining in intact diploid fibroblast cells derived from FA patients. FA fibroblasts from complementation groups A, C, D2, and G rejoined linearized plasmids with a significantly decreased efficiency compared to non-FA fibroblasts. Retrovirus-mediated expression of the respective FA cDNAs in FA cells restored their end-joining efficiency to wild type levels. Human FA fibroblasts and fibroblasts from FA rodent models were also significantly more sensitive to restriction enzyme-induced chromosomal DNA double-strand breaks than were their retrovirally-corrected counterparts. Taken together these data show that FA fibroblasts have a deficiency in both extrachromosomal and chromosomal DNA double-strand break repair, a defect that could provide an attractive explanation for some of the pathologies associated with FA.

INTRODUCTION

Fanconi anemia (FA) is a fatal inherited autosomal recessive disease characterized by progressive bone marrow failure and a significant predisposition toward malignancies, particularly acute myelogenous leukemia (1-2). Somatic-cell hybridization studies have shown that abnormalities in multiple genes result in FA (3-5). To date, at least eight distinct complementation groups have been identified (A, B, C, D1, D2, E, F, and G), and all of the known FA genes have been cloned (6-12). With the exception of \textit{FANCB}, and \textit{FANCD1}, none of the FA genes contain sequence motifs of known function (12), and only \textit{FANCD2} has been found to have a homolog in lower eukaryotic organisms (5). Some of the FA genes encode proteins that interact to form a complex in the nucleus. This complex is disrupted in cell lines from complementation groups A, C, E, F, and G (13-14). Yet despite these findings, the exact biological functions of the FA proteins
have not yet been determined and the molecular mechanism(s) responsible for FA have remained obscure (15).

Interestingly, although the molecular defect responsible for FA is unclear, it has been well documented that cells from FA patients display elevated levels of spontaneous chromosomal breaks and deletions, and have an increased sensitivity to the cytotoxic and clastogenic effects of DNA cross-linking agents (16-19). Patient-derived FA lymphoblasts have been shown to have significantly decreased plasmid rejoining fidelity compared to normal lymphoblasts (20-21). Additionally, nuclear extracts from patient-derived FA fibroblasts have substantially decreased plasmid rejoining activity compared to extracts from normal fibroblasts (22). These cellular features, along with the high susceptibility of FA patients to cancers, has lead to the hypothesis that this disorder results from defective DNA repair. However, the absence of recognizable DNA binding sequence motifs in FA genes, and the lack of evidence showing direct interaction of FA gene products with DNA, casts doubt on the idea that FA proteins directly participate in DNA repair. Recent results demonstrating a connection between the BRCA1 and BRCA2 tumor suppressor and FA proteins suggests that FA proteins may play an essential role in regulating the cellular response to DNA damage (12,15,23-25).

We examined both extrachromosomal and chromosomal DNA repair in intact FA fibroblasts and retrovirally-corrected FA fibroblasts from multiple complementation groups and animal models. We found that fibroblasts derived from FA patients of complementation groups A, C, D2, and G were significantly deficient in the repair of both plasmid and chromosome DNA double-strand breaks, and that this deficiency was corrected by expression of the respective FA cDNAs in these cells. Furthermore, a similar defect in DNA double-strand break repair was seen in cells derived from two rodent models of FA, and in wild type cells expressing a dominant negative FANCC allele.
EXPERIMENTAL PROCEDURES

Cell Culture—Cells were maintained in a humidified 5% CO₂-containing atmosphere at 37°C. All FA cell lines were obtained from the Oregon Health Sciences University unless otherwise noted. HT1080 are immortalized human sarcoma transformed fibroblast cells. MCF-7 (ATCC Cell Repository) and MA148 are human immortalized non-FA epithelial cells. CCL75.1, GM637, GM638, GM847 and GM10603 (NIGMS Human Genetic Cell Repository) are immortalized human non-FA fibroblasts. PD.715.F and PD.792.F are normal human diploid fibroblasts. PD.220i, PD.20i, and PD.20hygro (referred to as A’, D’, and D’”) are human immortalized FA fibroblasts of complementation groups A, D2, and D2 respectively. PD.20hygro:RV (referred to as D”-corrected) are PD.20hygro cells that have been infected with the retrovirus that expresses the FANCD2 cDNA. PD.720.F, 551-FAA, and PD.352.F (referred to as A”, C, and G) are human diploid FA fibroblasts from complementation groups A, C, and G respectively. 720-Retro, 551-FAC, and 352-FAG (referred to as A”-corrected, C-corrected, and G-corrected) are human primary FA fibroblasts that have been infected with retroviruses that express the FANCA, FANCC, and FANCG cDNAs respectively. Murine MPF60T, MPF61T, and MPF62T cells are embryonic fibroblasts derived from mice homozygous for Fancc[exon9], heterozygous for Fancc[exon9], and homozygous wild type respectively (26). Genotypes of these cells were verified by PCR analysis of genomic DNA. Chinese hamster ovary cells AA8 and UV40 (Xrcc9 mutants) were kindly provided by Dr. Larry H. Thompson, Lawrence Livermore National Laboratory, Livermore, CA (27). The patient-derived wild type and L554P FANCC alleles were kindly provided by Dr. Maureen E. Hotline, Oregon Health Sciences University and Portland Veterans Affairs Medical Center, Portland, OR (28).

DNA End-Joining—Experiments were carried out as previously described (20) with the following exceptions. Plasmid pSV2Neo was used as the substrate DNA. It was treated with the endonucleases EcoR I to produce cohesive DNA ends and Sma I to produce blunt ends. Linear
plasmid was gel-purified and quantitated. Five micrograms of plasmid pRSVEdl884 that encodes the SV40 large T-antigen was co-transfected into cells along with 1.25 μg of pSV2Neo. Plasmid DNA recovered after \( Dpn \) I digestion was electroporated into DH10B electrocompetent bacteria.

**Restriction Enzyme Electroporation**—Approximately \( 10^4 \) fibroblasts were collected and resuspended in 200 μl serum-free media along with restriction enzymes \( Pvu \) II or \( Hinf \) I (GibcoBRL, Carlsbad, CA) diluted in their respective storage buffers to concentrations of 0, 10, 20, 40, and 60 units per 50 μl. For heat inactivation, enzyme was diluted to the appropriate concentration and incubated for 18 hours at 70°C. The cell/enzyme mixture was electroporated with a BTX ECM 630 electroporator (Genetronic Inc., San Diego, CA) at an electrical field strength of 0.75 kV/cm and a capacitance of 960 μF. Cytotoxicity was examined using the sulforhodamine B (SRB) assay (29) or clonogenic assay. For the SRB assay, electroporated cells were plated into 24-well dishes followed by incubation for 24 hours. Percent cell survival was determined by comparing the optical density at 564nm for cells electroporated with 0 units of enzyme to the optical density for cells electroporated with 10-60 units of enzyme. For the clonogenic assay, electroporated cells were plated into 100mm dishes and allowed to grow and form colonies for 2-3 weeks. The number of colonies from electroporation with 0 units of enzyme was compared to the number of colonies obtained from electroporation with 10-60 units of enzyme.

**Restriction Enzyme Poration Mediated by Streptolysin O—\( Pvu \) II** was introduced into cells by poration with streptolysin O as previously described (30). Immediately afterward, cells were plated into 24-well dishes, incubated for 24 hours, and percent survival determined by the SRB assay as previously stated.

**Green Fluorescent Protein Electroporation**—Twenty-five micrograms of recombinant green fluorescent protein (Clontech, Palo Alto, CA) was electroporated into cells. After 6 hours, cells were examined by confocal microscopy. Uptake of the green fluorescent protein was determined by
comparing the number of cells fluorescing at 522 nm (excitation 488 nm) to the number of cells observed in the bright field.

RESULTS

Plasmid end-joining in intact wild type and FA fibroblasts—DNA end-joining was examined within intact fibroblast cells by electroporating linear plasmid DNA molecules into fibroblasts. Rejoined plasmids were recovered from fibroblasts and analyzed with a bacterial reporter system. The frequency with which the linearized DNA was rejoined was determined by comparing the number of bacterial colonies obtained when fibroblasts were electroporated with linear plasmid DNA to the number obtained when fibroblasts were electroporated in a parallel experiment with circular plasmid DNA (20). Examination of a number of wild type cell lines revealed that plasmids with cohesive DNA ends were rejoined with approximately 28% efficiency and that there were no significant differences amongst any of the cells examined (Table I). In contrast, the rejoining efficiency of the same plasmid substrate was only approximately 4% in fibroblasts derived from FA patients from complementation groups A, C, D2, and G (Table I). Additional experiments using blunt-ended plasmid DNA revealed similar results; FA fibroblasts of complementation groups A, C, D2, and G had rejoining frequencies with blunt-ended substrate of 3.0%, 5.1%, 3.1%, and 4.4%, respectively, while the efficiency of rejoining of these substrates in non-FA cells was approximately 30%. To verify that all rejoined plasmids recovered by the bacterial reporter system were derived exclusively from substrates processed within human cells, both cohesive-ended and blunt-ended linearized plasmid substrate was electroporated directly into bacteria. As expected, no bacterial colonies were obtained from this control experiment (data not shown).

Interestingly, we had previously found that nuclear extracts prepared from fibroblasts derived from 8 unrelated normal human donors rejoined linear plasmids with an average efficiency of 26%, while extracts from fibroblasts derived from three unrelated FA patients rejoined these plasmids with efficiencies ranging from 4% to 12% (22). Analysis revealed that the majority of
products from both the cell-free and intracellular rejoining reactions were precisely rejoined, while a substantial minority had suffered small deletions that spanned direct sequence repeats.

Plasmid rejoining was also examined in FA cell strains that had been ‘corrected’ through infection by retroviruses encoding the corresponding FA cDNA. It has previously been demonstrated that such correction restored resistance to crosslinking agents in these FA cell strains (6, 8-11). As Table I reveals, in all four cases the retrovirally-corrected FA cells rejoined cohesive-ended DNA with frequencies similar to that seen in non-FA cells. Additionally, the end-joining efficiency of blunt-ended DNA substrate was approximately 30% in these cells. As was expected, infection of an FANCC cell strain with a retrovirus encoding an \textit{FANCA} cDNA had no effect on plasmid rejoining efficiency (data not shown).

\textit{Hypersensitivity of FA fibroblasts to restriction enzyme-induced cell death—}Cells cultured from FA patients display an increased level of chromosomal abnormalities, including spontaneous breaks and deletions (1). This, along with the severe plasmid rejoining defect seen in FA fibroblasts, prompted us to hypothesize that FA fibroblasts would be sensitive to induced chromosomal DNA double-strand breaks. To test this hypothesis, intact FANCD2 cells and their retrovirus-corrected counterparts were electroporated with restriction endonucleases. This treatment has been shown to create chromosomal DNA double-strand breaks, ultimately resulting in cell death (31-35).

FANCD2 fibroblasts electroporated with either \textit{Pvu} II, which creates blunt-ended double-strand breaks (Fig. 1A), or \textit{Hinf} I, which creates cohesive-ended double-strand breaks (Fig. 1B), were significantly more sensitive to restriction enzyme-induced cell death than their retrovirus-corrected counterparts. Heat inactivation of both \textit{Pvu} II and \textit{Hinf} I prior to electroporation abolished their ability to induce cell death (Fig. 1). Cell viability following these restriction enzyme treatments was determined using the sulforhodamine B (SRB) assay (29). To ensure that this assay accurately measured cytotoxicity, analogous experiments were analyzed using clonogenic assays. A similar decrease in survival was seen in FANCD2 fibroblasts compared to retrovirally-corrected FANCD2 cells when survival was determined through colony formation (data not shown). To rule out the
possibility that the decreased cell survival of FA fibroblasts was caused by the deleterious affects of electroporation, streptolysin O treatment was used to introduce Pvu II into cells (30). A decrease in cell survival similar to that observed following enzyme electroporation was seen after streptolysin O-mediated poration of Pvu II (data not shown). Additionally, the SRB and clonogenic assays both showed that cell death due to poration alone was comparable in FA and non-FA cells following electroporation and streptolysin O-mediated poration, resulting in approximately 40-60% killing in all cells examined.

As Table II reveals, while FA fibroblast cells from complementation groups A, C, D2, and G displayed increased restriction enzyme-induced cell death, their retrovirus-corrected counterparts had sensitivities similar to non-FA cells. To ensure that the reduced sensitivity of retrovirus-corrected FA cells to restriction enzymes was not due to a difference in transfection efficiency of porated protein, we examined the extent to which they and their unmodified counterparts took-up recombinant green fluorescent protein. We found that 69.8 +/- 2.4% of uncorrected FANCD2 cells accumulated significant levels of green fluorescent protein following electroporation, compared to 71.2 +/- 1.1% of the corrected cells. Since both FA and retrovirus-corrected FA fibroblasts are able to take-up similar amounts of electroporated protein, the observed sensitivity to restriction enzyme-induced cell death is not due to increased restriction enzyme uptake. It is noteworthy that the percent of cells taking-up electroporated green fluorescent protein is roughly similar to the number of FA cells killed after restriction enzyme poration. This result suggests that all of the FA cells that take-up restriction enzymes are killed while the non-FA cells and corrected FA cells that take-up restriction enzymes are relatively resistant to their cytotoxic affects.

**DNA end-joining and chromosomal double-strand break sensitivity in HT1080 cells expressing a dominant negative FANCC allele**—The finding that overexpression of a patient-derived FANCC allele (L554P) rendered normal cells sensitive to the crosslinking agent diepoxybutane (28) prompted us to ask whether overexpression of this dominant negative allele in HT1080 cells would also render them deficient in plasmid rejoining and hypersensitive to
restriction enzyme-induced cell death. As Fig. 2A indicates, transgenic HT1080 fibroblasts expressing the L554P FANCC allele were hypersensitive to diepoxybutane. Furthermore, these cells rejoined both cohesive-ended and blunt-ended linearized plasmids with significantly diminished efficiency (Fig. 2B). HT1080 cells expressing L554P were also hypersensitive to restriction enzyme-induced cell death following electroporation of Pvu II (Fig. 2C). The decreases in plasmid end-joining and cell survival following restriction enzyme treatment were of similar magnitude to those observed in the patient-derived FA fibroblasts of complementation groups A, C, D2, and G. Overexpression of the wild type FANCC allele in HT1080 cells had no effect on any of the parameters examined (Fig. 2A-C).

Restriction enzyme-induced cell death in fibroblasts from two rodent models of FA—We next examined whether the hypersensitivity to restriction enzyme-induced cell death seen in human FA fibroblasts was also observed in two rodent models of FA. Embryonic fibroblasts derived from mice homozygous for a targeted deletion of exon 9 of the murine FA complementation group C gene (Fancc<sup>exon9</sup>) display chromosomal breaks and are hypersensitive to DNA crosslinking agents in a manner similar to human FA cell strains (26). As Fig. 3A indicates, these murine fibroblasts were also hypersensitive to restriction enzyme-induced cell death while wild type murine embryo fibroblasts and murine embryo fibroblasts heterozygous for the deleted Fancc allele were resistant. Likewise, Chinese hamster ovary-derived UV40 cells (27), which fail to produce functional Xrcc9 protein (the hamster homolog of human FANCG protein) and have also been shown to be hypersensitive to DNA crosslinking agents (36), were significantly more sensitive to restriction enzyme-induced cell death than were cells of the wild type parental cell line AA8 (Fig. 3B). These data indicate that the FA-like rodent cells are as sensitive to restriction enzyme-induced killing as human FA fibroblasts.
DISCUSSION

The data presented herein support the conclusion that FA fibroblasts have a dramatically reduced ability to rejoin double-strand breaks in both introduced plasmids, as well as within their chromosomes. These defects were observed in diploid fibroblasts from patients whose cells belong to a number of different FA complementation groups. In all cases tested, re-introduction of the deficient FA gene into these cells eliminated the aberrant phenotype. An inability to repair restriction enzyme-induced chromosomal double-strand breaks was also observed in two different rodent models of FA, as well as in a human cell culture model of FA induced by overexpression of a dominant negative allele of the \textit{FANCC} gene. Taken together these findings provide robust support for the conclusion that FA fibroblasts have a defect in cellular DNA double-strand break repair.

The nature of the DNA double-strand break repair defect in FA fibroblasts however remains obscure. It is known that mammalian cells utilize both recombinational and non-homologous end-joining pathways to repair DNA double-strand breaks. Thus, in principle either mechanism, or conceivably both, could be affected in FA fibroblasts. The linearized plasmid substrates utilized in our end-joining assays cannot be repaired \textit{via} a recombinational mechanism. Thus we can conclude that FA fibroblasts have a defect in a non-recombinational DNA double-strand break repair pathway. The findings that V(D)J recombination and Ku-dependent non-homologous end-joining are not affected in FA cells (20-22, 37), indicate that this observed defect does not involve that pathway. Instead it appears that the deficiency resides in another, currently uncharacterized non-recombinational repair pathway.

It is tempting to speculate that the hypersensitivity of FA fibroblasts to restriction enzyme-induced chromosomal double-strand breaks is a consequence of deficient non-recombinational repair of these lesions. However, a number of findings suggest that this hypersensitivity could reflect a deficiency in chromosomal homologous recombinational repair. First, the BRCA1 protein, which is required for efficient recombinational repair of chromosome double-strand breaks, co-localizes to nuclear foci with the FANCD2 protein in cells following exposure to ionizing radiation
FANCD2 has also been shown to be phosphorylated in an ATM-dependent manner following induced DNA damage (25). Second, the FANCB and FANCD1 genes are apparently identical to BRCA2, a gene also required for efficient recombinational repair of chromosome double-strand breaks (12, 15, 38). Third, both the BRCA1 and BRCA2 proteins interact with the mammalian RecA homolog Rad51 (39), and cells deficient in BRCA1 and/or BRCA2 have a significant defect in homologous recombination, display chromosomal instability, and are hypersensitive to DNA cross-linking agents as are FA cells (38, 40-45). It is thus conceivable that FA cells have a defect in recombinational repair of chromosomal DNA double-strand breaks. It remains to be determined whether the elevated sensitivity of FA cells to restriction enzyme-induced cell death is a consequence of defective non-homologous end-joining, defective recombinational repair, or both. It may be possible to gain insight into this question by studying the repair of double-strand breaks induced into engineered chromosomal loci by rare-cutting endonucleases such as the yeast I Sce-I enzyme.

An alternative explanation for the cytotoxicity observed in FA fibroblasts following introduction of restriction enzymes is that cell death may be due to improper checkpoint regulation following chromosomal damage. A recent report by Taniguchi et al. shows that FANCD2 fibroblasts have radioresistant DNA synthesis following induced DNA damage (25). Similarly, BRCA2/FANCD1-deficient Chinese hamster ovary cells and BRCA1-deficient cells also display radioresistant DNA synthesis after exposure to ionizing radiation (46-47). Thus, given the association between FA proteins and BRCA1 and BRCA2 previously outlined, these data indicate that FA cells may have an S-phase checkpoint defect. Failed repair of DNA double-strand breaks and in inability to regulate an essential checkpoint may result in these FA cells progressing through the cell cycle with unrepaired chromosomal lesions that would ultimately lead to cell death.

Regardless of the nature of the defect, the deficiency in DNA double-strand break repair observed in FA fibroblasts may provide an attractive explanation for some of the pathologies associated with FA. Although this conclusion is based on studies performed on fibroblast cells and may not be applicable to all cell types, evidence from lymphoblasts derived from FA patients also
indicates a deficiency in DNA double-strand break repair (20-21, 48). Furthermore, examination of both fibroblasts and lymphoblasts derived from FA patients has revealed no distinct differences in sensitivities to DNA damaging agents or chromosomal instability, the two main cellular features of FA (16, 19, 49-50). Thus the cancer-predisposition that characterizes this disorder could result from chromosomal rearrangements due to defective repair of chromosome double-strand breaks that arise spontaneously or are created as intermediates in normal cellular processes. Likewise, just as defective repair of spontaneous DNA double-strand breaks caused by oxygen is responsible for the neuronal apoptosis observed in knockout mice lacking a functional non-homologous end-joining pathway (51), the defective DNA double-strand break repair we observe in FA cells could be responsible for the bone marrow failure observed in these patients. Therefore, one exciting possibility is that pharmacological approaches may be developed to activate the defective DNA double-strand break repair pathway in FA cells. Such therapeutic intervention could potentially halt the inexorable loss of bone marrow stem cells that results in fatal anemia in these patients, thereby extending their lifespan.

Acknowledgements—This work was supported by National Institutes of Health grant (AG16678) and the Breast Cancer Research Program grant (DAMD17-99-1-9299) from the US Department of Defence. We would like to thank Drs. Larry H. Thompson, Maureen E. Hoatlin, and Barbara Cox for kindly providing reagents used herein.

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FIGURE LEGENDS

**Fig. 1: FA fibroblasts are sensitive to restriction enzyme-induced chromosomal double-strand breaks.** A, FANCD2 fibroblasts (○) and retrovirus-corrected FANCD2 fibroblasts (□) were electroporated in the presence of various amounts of *Pvu* II restriction enzyme and their percent survival was determined. Percent survival was also determined following electroporation of heat inactivated *Pvu* II enzyme into FANCD2 fibroblasts (□) and retrovirally-corrected fibroblasts (○). B, Identical treatments of FANCD2 and their retrovirus-corrected counterparts were performed using the restriction enzyme *Hinf* I (symbols are the same as in part A).

**Fig. 2: HT1080 fibroblasts expressing the patient-derived mutant FANCC allele L554P resemble FA cells.** A phenotypic analysis was performed on HT1080 cells (black bars), as well as on HT1080 cells that over-expressed a wild type FANCC allele (shaded bars), and HT1080 cells that over-expressed the L554P FANCC allele (white bars). Data represent the mean of three experiments, error bars depict the SEM, *p<0.005. A, Sensitivity to diepoxybutane. B, Plasmid rejoining efficiency. C, Sensitivity to restriction enzyme-induced DNA double-strand breaks.

**Fig. 3: Mouse fibroblasts deficient in Fancc are sensitive to restriction enzyme-induced DNA double-strand breaks.** Data represent the mean of three experiments, error bars depict the SEM, *p<0.005. A, restriction enzyme-induced cell death was measured in mouse embryonic fibroblasts homozygous for an Fancc exon 9 deletion allele (○), normal mouse fibroblasts (□), and mouse fibroblasts heterozygous for the Fancc exon 9 deletion allele (□). B, restriction enzyme-induced cell death was measured in Xrcc9-deficient UV40 cells (□), and the UV40 parental cell line AA8 (□).
Table I: DNA end-joining efficiency in FA and corrected-FA fibroblasts

| Cells          | FA group | Rejoining efficiency (%)<sup>a</sup> |
|---------------|----------|-------------------------------------|
| HT1080        | WT       | 29.8 ± 8.3                          |
| MCF-7         | WT       | 28.9 ± 3.9                          |
| MA148         | WT       | 37.4 ± 9.2                          |
| CCL75.1       | WT       | 27.1 ± 7.3                          |
| GM637         | WT       | 29.2 ± 2.6                          |
| GM638         | WT       | 24.9 ± 1.2                          |
| GM847         | WT       | 27.6 ± 0.9                          |
| GM10603       | WT       | 26.6 ± 1.2                          |
| PD.715.F      | WT       | 25.9 ± 5.4                          |
| PD.792.F      | WT       | 27.8 ± 6.1                          |
| FANCAi        | A'       | 4.7 ± 2.1                          * |
| PD.720.F      | A"       | 6.1 ± 3.1                          * |
| 551-FAA       | C        | 4.2 ± 2.9                          * |
| FANCDi        | D'       | 3.9 ± 3.0                          * |
| FANCDnc       | D"       | 6.2 ± 1.0                          * |
| PD.352.F      | G        | 5.4 ± 2.0                          * |
| 720-Retro     | A"-corrected | 29.2 ± 2.3                      |
| 551-FAC       | C -corrected | 27.6 ± 1.7                      |
| FANCDc        | D"-corrected | 28.1 ± 5.3                      |
| 352-FAG       | G -corrected | 25.0 ± 4.9                      |

<sup>a</sup>Data represent the mean percent survival from at least three experiments in each cell strain and cell line, *p<0.005.
# Table II: Decreased survival of FA cells following Pvu II electroporation

| Cells                  | FA group | Rejoining efficiency (%)<sup>a</sup> |
|------------------------|----------|-------------------------------------|
|                        |          | 10 U Pvu II | 20 U Pvu II |
|                        |          |            |            |
| HT1080                 | WT       | 94.1 ± 6.6 | 92.3 ± 1.1 |
| MCF-7                  | WT       | 99.6 ± 3.4 | 96.8 ± 2.8 |
| MA148                  | WT       | 99.6 ± 0.6 | 99.3 ± 1.2 |
| CCL75.1                | WT       | 99.3 ± 1.3 | 100 ± 0.0  |
| GM637                  | WT       | 98.5 ± 2.7 | 91.3 ± 8.1 |
| GM638                  | WT       | 97.1 ± 3.5 | 100 ± 0.0  |
| GM847                  | WT       | 95.7 ± 3.8 | 97.2 ± 4.9 |
| GM10603                | WT       | 98.2 ± 1.7 | 95.2 ± 8.3 |
| PD.715.F               | WT       | 98.6 ± 3.0 | 97.9 ± 2.1 |
| PD.792.F               | WT       | 95.2 ± 7.0 | 93.1 ± 3.2 |
| FANCAi                 | A'       | 75.9 ± 16.6| 40.2 ± 8.0 | * |
| PD.720.F               | A''      | 81.4 ± 7.3 | 66.7 ± 11.6| * |
| 551-FAA                | C        | 82.6 ± 7.7 | 65.1 ± 4.4 | * |
| FANCDi                 | D'       | 92.1 ± 3.6 | 43.4 ± 2.8 | * |
| FANCDnc                | D''      | 91.1 ± 3.3 | 45.3 ± 1.1 | * |
| PD.352.F               | G        | 83.7 ± 3.1 | 56.9 ± 9.1 | * |
| 720-Retro              | A''-corrected | 95.9 ± 2.1 | 94.1 ± 9.4 |
| 551-FAC                | C -corrected | 90.9 ± 12.9| 96.7 ± 5.8 |
| FANCDc                 | D''-corrected | 97.6 ± 8.4 | 90.1 ± 4.1 |
| 352-FAG                | G -corrected | 98.7 ± 2.2 | 98.3 ± 2.9 |

<sup>a</sup>Data represent the mean percent survival from at least three experiments in each cell line and cell strain, *p<0.005.
Figure 1
Figure 2
Figure 3
A DNA double-strand break repair defect in fanconi anemia fibroblasts
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J. Biol. Chem. published online October 1, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M207937200

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