THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 46, Issue of November 12, pp. 32904–32908, 1999
Printed in U.S.A.
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Human α Spectrin II and the Fanconi Anemia Proteins FANCA and FANCC Interact to Form a Nuclear Complex*

(Received for publication, May 13, 1999, and in revised form, August 5, 1999)

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Fanconi anemia (FA) is a genetic disorder characterized by bone marrow failure, congenital abnormalities, cancer susceptibility, and a marked cellular hypersensitivity to DNA interstrand cross-linking agents, which correlates with a defect in ability to repair this type of damage. We have previously identified an approximately 230-kDa protein present in a nuclear protein complex in normal human lymphoblastoid cells that is involved in repair of DNA interstrand cross-links and shows reduced levels in FA-A cell nuclei. The FANCA gene appears to play a role in the stability or expression of this protein. We now show that p230 is a well known structural protein, human α spectrin II (αSpIIΣ*), and that levels of αSpIIΣ* are not only significantly reduced in FA-A cells but also in FA-B, FA-C and FA-D cells (*i.e.* in all FA cell lines tested), suggesting a role for these FA proteins in the stability or expression of αSpIIΣ*. These studies also show that αSpIIΣ* forms a complex in the nucleus with the FANCA and FANCC proteins. αSpIIΣ* may thus act as a scaffold to align or enhance interactions between FA proteins and proteins involved in DNA repair. These results suggest that FA represents a disorder in which there is a deficiency in αSpIIΣ*.

Fanconi anemia (FA)[1] is a recessively transmitted genetic disorder characterized by bone marrow failure, diverse congenital abnormalities, and an increased incidence of cancer (1–3). One of the distinguishing characteristics of this disease is the marked cellular hypersensitivity to interstrand cross-linking agents, which correlates with a defect in ability to repair damage produced by these agents (3–7). The etiopathogenesis of FA remains unclear, however, despite the fact that genes for three of its eight known complementation groups (*FANCA*, *FANCC*, and *FANCG*) have been cloned and their protein products examined (8–11). How the DNA repair defect is related to the FA genes or gene products or to the clinical manifestations of the disorder is unclear.

We have isolated a chromatin-associated protein complex from the nuclei of normal human lymphoblastoid cell nuclei and have shown that this complex is involved in the repair of DNA interstrand cross-links (12–15). A number of the proteins involved in nucleotide excision repair are present in this complex (16). In FA-A and FA-D cells there is a defect in the ability of this complex to incise DNA containing interstrand cross-links (13, 14). We have recently shown that there is a deficiency in FA-A cells in the levels of an approximately 230-kDa protein present in this nuclear complex and that the *FANCA* gene plays a role in the stability or expression of this protein (16).

We have now determined the identity of the 230-kDa protein and shown that it is the structural protein α spectrin II (αSpIIΣ*). We have also shown that levels of αSpIIΣ* are significantly reduced in FA-B, FA-C, and FA-D, as well as FA-A cells and that this protein forms a complex in the nucleus with the FANCA and FANCC proteins. Thus αSpIIΣ* may act as a scaffold to align or enhance interactions between the FA proteins and other proteins in the nucleus such as those involved in DNA repair. Because nonerythroid α spectrin has been shown to interact with proteins involved in a number of cellular processes, such as DNA synthesis, cell cycle progression, gene expression, signal transduction, and cell growth and differentiation (17–22), a deficiency in this protein in FA cells could have far reaching consequences due to the number of systems affected. This may explain some of the diverse cellular and clinical defects that have been reported in FA (1–3).

EXPERIMENTAL PROCEDURES

Chromatin-associated Protein Extracts—Normal human (GM 1989 and GM 3299) lymphoblastoid cell lines were obtained from the Coriell Institute for Medical Research, Camden, NJ. FA-A (HSC 72 and HSC 99), FA-B (HSC 230), FA-C (HSC 536), and FA-D (HSC 62) lymphoblastoid cell lines were a gift from Dr. Manuel Buchwald (Hospital for Sick Children, Toronto, Canada). FA-A lymphoblastoid cells (HSC 72) were stably transduced with a retroviral vector expressing the *FANCA* cDNA (HSC 72–17) (23). Cell lines were grown in suspension culture in RPMI 1640 medium as described previously and routinely checked for mycoplasma using an American Type Culture Collection polymerase chain reaction-based mycoplasm detection kit (13). HeLa cells were obtained from Cellex Biosciences, Inc., Minneapolis, MN. Cell nuclei were isolated, and the chromatin-associated proteins were extracted from them in a series of steps as described previously (13, 24).

Development and Purification of Monoclonal antibodies (mAb)—mAbs were developed, as described previously, against proteins in the endonuclease complex, pI 4.6, which was isolated from chromatin-associated proteins in the nuclei of normal human lymphoblastoid cells and recognizes and incises DNA containing interstrand cross-links (12, 16). One of the mAbs developed was against the 230-kDa protein. This mAb, which completely inhibited activity of the protein complex on DNA containing interstrand cross-links produced by 8-methoxypsoralen plus UVA light, was of the IgM class; it was subsequently purified on a Superose 6HR 10/30 size exclusion column (Amersham Pharmacia Biotech).

Identification of the 230-kDa Protein—HeLa chromatin-associated proteins were separated by SDS-PAGE on a 7–9.5% gradient gel (15 × 20-cm). After separation, a portion of the gel was cut off and electroblotted onto a nitrocellulose membrane. One-half of the membrane was

* This work was supported by National Institutes of Health Grants R01 HL54806 (to M. W. L.) and CA09665 (to L. W. M.) and by a Translational Leukemia of America Award (to C. E. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: FA, Fanconi anemia; mAb, monoclonal antibody; IP, immunoprecipitation; MALDI-MS, matrix-assisted laser desorption ionization mass spectrometry; PAGE, polyacrylamide gel electrophoresis.

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stained with colloidal gold (Bio-Rad), and the other half was immunoblotted with anti-p230. In this way the 230-kDa band could be identified on the stained membrane. The remainder of the gel was stained with Coomassie, and the 230-kDa band was identified, excised, and sent to the HHMI Biopolymer Facility/W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University for mass spectrometric analysis by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS).

At the Keck Facility, the protein was digested within the gel slice with trypsin. The resulting digest was mixed with α-cyano-4-hydroxy cinnamic acid matrix solution and spotted onto a sample target (25). The sample target was then introduced into the mass spectrometer, a Micromass TofSpec S.E. that can be used in either the linear or reflectron mode and is equipped with delayed extraction, which increases resolution and mass accuracy. The resulting peptide masses were then subjected to peptide mass searching using Peptide Search and Profound to identify proteins whose sequences were in the EMBL nonredundant and OWL data bases, respectively.

Staining of Electrophoretically Separated HeLa Cell Proteins—To get a better idea of the relative abundance of the 230-kDa protein in the HeLa cell chromatin-associated protein extracts, these extracts were separated by SDS-PAGE according to the method of Laemmli (26). Samples were electrophoresed on two different 15 × 20-cm gels, each with a 4% stacking gel: one using a 7–9.5% gradient (to maximize separation of the higher molecular weight range proteins), and the other using a 12.5–15% gradient (for separation of the lower molecular weight proteins). In addition, human erythroid spectrin (Sigma) was also electrophoresed on the 7–9.5% gradient gel. Two different molecular weight markers were run alongside these lanes: NOVEX See Blue Ladder (4–250-kDa range) and Bio-Rad Kaleidoscope Ladder (7.1–208-kDa range). After electrophoresis, gels were stained with SYPRO orange (1:5,000 dilution) (Bio-Rad) in 10% acetic acid for 45 min to 1.5 h. Gels were visualized on a VISTA FluorimagerSI using ImageQuant software (Molecular Dynamics).

Immunoprecipitation—For immunoprecipitation (IP), chromatin-associated protein extracts from normal human, HeLa, FA-A, and transduced FA-A cells were prepared in 50 mM potassium phosphate, pH 7.0, 1 mM EDTA, 1 mM dithiothreitol, and 40% glycerol. For anti-FANCA IP, affinity-purified rabbit polyclonal antisera generated from the carboxyl-terminal region of the FANCA protein, or pre-immune serum, was bound to protein A-coated agarose beads (Sigma) in binding buffer (25 mM Tris-Cl, pH 7.3, 150 mM NaCl, 1% Triton X-100 plus protease inhibitor mixture (Roche Molecular Biochemicals)). For anti-α spectrin IPs, anti-α spectrin (prepared against chicken blood cell membranes, specific for mammalian nonerythroid α spectrin and chicken α spectrin) (Chemicon, mAb 1622), or anti-mouse IgG_1 (Sigma) (used as a control) was bound to protein G-coated agarose beads (Sigma) in binding buffer. These beads were blocked in blocking buffer (4% nonfat dried milk blocker (Bio-Rad) in TTBS (25 mM Tris-Cl, pH 7.3, 150 mM NaCl, and 0.05% Tween 20)), washed, and resuspended as a 1:3 slurry in binding buffer. Immunoprecipitation was carried out by mixing 30 μl of this slurry with 25 μg of chromatin-associated protein extract and an equal volume of IP buffer (25 mM Tris-Cl, pH 7.3, 125 mM NaCl, 1% Triton X-100 plus protease inhibitor mixture) overnight at 4 °C. Protein concentrations were determined using Bradford reagent (Bio-Rad). The protein A- or G-agarose bead-bound immune complexes were then washed three times with IP buffer and resuspended in this buffer. The IPs and aliquots of chromatin-associated extracts from the cell lines examined were subjected to SDS-PAGE, using 8–16% gradient gels or 10% gels. After electrophoresis, the proteins were transferred to nitrocellulose and immunoblotted.

Immunoblotting—For immunoblotting, chromatin-associated proteins from the nucleus of either normal human lymphoblastoid, HeLa, FA-A, FA-B, FA-C, FA-D, or transduced FA-A cells, or human erythroid spectrin (Sigma) were separated on SDS-PAGE, using either 7.5 × 8.5-cm or 15 × 20-cm gels, following the method of Laemmli (26). The proteins were then transferred to nitrocellulose in transfer buffer (25 mM Tris-Cl, pH 8.3, 192 mM glycine, and 20% (v/v) methanol) at 100 volts for 2 or 2.5 h at 4 °C. The membranes were blocked in blocking buffer for 1 h at room temperature or overnight at 4 °C and then incubated in primary antibody in TTBS overnight at 4 °C. After washing in TTBS, the blots were incubated in secondary antibody (anti-mouse IgM, anti-mouse IgG, or anti-rabbit IgG) or protein A conjugated to horseradish peroxidase in 1% nonfat dried milk blocker (Bio-Rad) in TTBS. The blots were washed further in TTBS and reacted with substrate (Pierce Ultra or Pierce Super chemiluminescent substrate) and exposed to x-ray film (Pierce). The primary antibodies used were anti-αSpIIΣ*, anti-α spectrin, anti-FANCA (carboxyl-terminal), anti-FANCC (amino-terminal) (generous gift of Dr. Alan D'Andrea, Harvard Medical School), and anti-topoisomerase II (Oncogene). When blots were reprobed, they were stripped of IgM using a high salt buffer (20 mM Tris, pH 7.5, 500 mM NaCl, and 0.3% Tween 20) and IgG per the manufacturer's instructions (Pierce) and were reblocked before reprobing with primary antibody. Alternatively, blots were incubated with secondary antibody conjugated to alkaline phosphatase in 1% nonfat dried milk blocker (Bio-Rad) in TTBS, washed in TTBS and incubated in 5-bromo-4-chloro-3-indoyl phosphate/nitro blue tetrazolium solution (Bio-Rad). Images were scanned using a Hewlett-Packard ScanJet 4c/T scanner and analyzed using ImageQuant (Molecular Dynamics).

RESULTS

A mAb developed against the 230-kDa protein has been used in its identification. This protein, after separation by gel electrophoresis and identification by Western blot analysis, was digested with trypsin and analyzed by MALDI-MS. The rationale for using this approach is that it is very sensitive and the mass spectrum of the peptide mixture, resulting from the enzymatic digestion of the protein, provides a fingerprint that can be specific enough to identify the protein (27).

Using this method, the 230-kDa protein was identified as human nonerythroid α spectrin (α spectrin II). The calculated peptide masses obtained from the protein digest were searched against two different data bases to determine the number of matched peptides. A ProFound search of the peptides obtained from the 230-kDa protein digest gave a probability score of 1.0e+00 to human α spectrin II with a clean break between this score and the score of the next nonrelated protein. The percentage coverage of the known sequence for this protein was 35% (*i.e.* the peptide masses obtained for analysis matched to 35% of the known protein sequence). This exceeded the minimum percentage coverage of 25% set as a criterion for a protein match by the Keck Facility. A second ProFound search was performed, after deleting sequences that matched human α spectrin II, and no additional protein was identified. PeptideSearch matched the same protein with 33% coverage. This analysis of the 230-kDa protein was repeated on a second sample obtained from another HeLa cell chromatin-associated protein extract with the same result. Because the isoform of the α spectrin we have identified is unknown, this protein has been designated αSpIIΣ* in accordance with proposed nomenclature (17, 28).

Confirmation of the 230-kDa protein as αSpIIΣ* was made by Western blot analysis. A mAb against an α spectrin, with specificity for mammalian nonerythroid α spectrin (Chemicon), bound to the 230-kDa protein from HeLa chromatin-associated protein extracts (Fig. 1, *lane 1*); binding of this mAb to human erythroid spectrin (α and β chains) (Fig. 1, *lane 2*) was used as a control. Similarly, anti-αSpIIΣ* bound to α spectrin in HeLa cell extracts and to the α and β chains of erythroid spectrin (Fig. 1, *lane 4*). Both anti-α spectrin and anti-αSpIIΣ* showed cross-reactivity with α and β spectrin. Using these antibodies, β spectrin was not detected in the HeLa cell chromatin-associated protein extracts. Confirmation that both mAbs were binding to the same protein band was made by analysis of the respective immunoblots lined up with gold-stained proteins from a section of the same membrane (data not shown). Further confirmation of the location of β spectrin on these gels was obtained by Western blot analysis using a mAb that principally recognized erythroid β spectrin (data not shown).

Electrophoretic separation of the proteins in the HeLa cell chromatin-associated protein extracts showed that αSpIIΣ* is not one of the major bands present in these protein extracts (Fig. 2, *A*, *lane 1* and *B*). The αSpIIΣ* band (Fig. 2*A*, *lane 1*) lined up exactly with the α spectrin band from erythroid spectrin (Fig. 2*A*, *lane 2*), which was run alongside it on the same gel. The identity of these bands was verified by Western blot analysis. Both proteins, according to electrophoretic mobility,

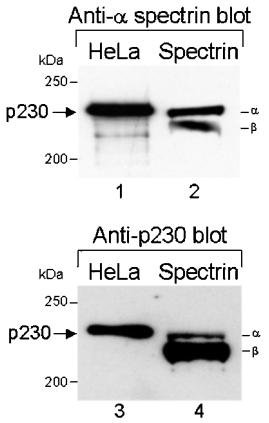

FIG. 1. **Confirmation of the identity of the 230-kDa protein as nonerythroid α spectrin.** Western blot analysis was carried out on the binding of anti-α spectrin (Chemicon) (1:8,000 dilution) (*lanes 1* and *2*) and anti-p230 (*lanes 3* and *4*) to chromatin-associated proteins from HeLa cells (*lane 1* and *3*) or to erythroid spectrin (*lanes 2* and *4*). The blot was first probed with anti-p230 and then stripped and reprobed with anti-α spectrin.

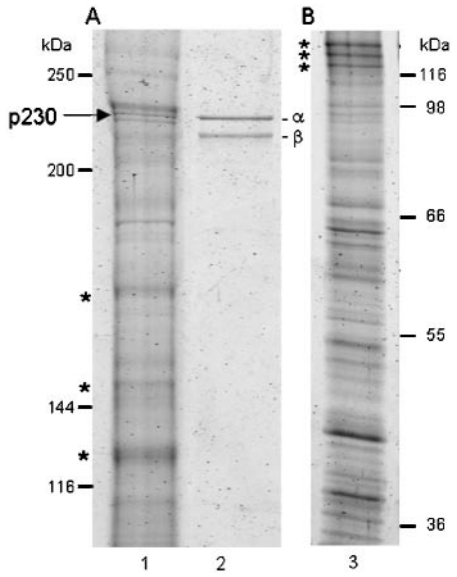

FIG. 2. **Electrophoretic separation of the HeLa cell chromatin-associated protein extracts.** The HeLa cell extracts were separated by SDS-PAGE on a 15 × 20-cm gel and stained with SYPRO orange. *A*, a 7–9.5% gradient gel for separation of proteins above 100 kDa. *Lane 1*, HeLa cell extract; *lane 2*, erythroid spectrin. *B*, a 12.5–15% gradient gel for separation of proteins below 100 kDa. *Asterisks* indicate the same bands on different gels.

had an apparent molecular mass of approximately 230 kDa, which agrees with previously reported values (230–240 kDa) (18, 29) and is lower than the calculated molecular mass of 284 kDa for nonerythroid and 280 kDa for erythroid α spectrin (18, 30).

Immunoblotting showed that there are reduced levels of the 230-kDa protein in chromatin-associated protein extracts from FA complementation group B (FA-B), C (FA-C), and D (FA-D) cells (Fig. 3, *top panel*, *lanes 3–5*) compared with normal extracts (Fig. 3, *top panel*, *lane 1*) just as there are in the FA-A extracts (Fig. 3, *top panel*, *lane 2*). Thus in at least four FA complementation groups there is a deficiency in αSpIIΣ*. In these studies topoisomerase II was used as an internal control (Fig. 3, *bottom panel*).

Levels of αSpIIΣ* are restored to normal in FA-A cells that have been corrected with *FANCA* cDNA (Fig. 4, *top panel*, *lanes 1–3*), as previously shown (16), indicating that the *FANCA* gene

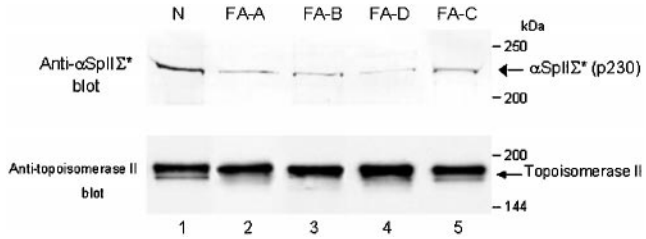

FIG. 3. **A deficiency in αSpIIΣ* in FA complementation groups A, B, C, and D.** Binding of anti-αSpIIΣ* to this protein in chromatin-associated protein extracts from (*top panel*) normal human lymphoblastoid (*lane 1*), FA-A (HSC 72) (*lane 2*), FA-B (HSC 230) (*lane 3*), FA-D (HSC 62) (*lane 4*), and FA-C (HSC 536) (*lane 5*) cells. The *arrow* indicates mAb binding to αSpIIΣ*. Binding of anti-topoisomerase II to topoisomerse II in these extracts (indicated by the *arrow*) was used as an internal control for protein loading (*bottom panel*, *lanes 1–5*).

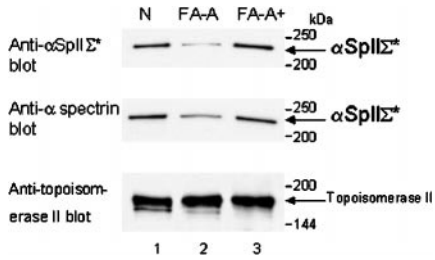

FIG. 4. **The deficiency in αSpIIΣ* in FA-A cells is restored in the corrected cells.** Western blot analysis was carried out on binding of anti-α SpIIΣ* (*top panel*) and anti-α spectrin (Chemicon) (*second panel*) to chromatin-associated proteins from the nuclei of normal human lymphoblastoid cells (*lane 1*), FA-A cells (HSC 72) (*lane 2*), and FA-A cells transduced with a retroviral vector expressing the *FANCA* cDNA (HSC 72–17) (*lane 3*). This blot was also probed with anti-topoisomerase II, which was used as a loading control (*bottom panel*, *lanes 1–3*).

plays a role in the stability or expression of this protein. Similar results were obtained when immunoblotting was carried out using anti-α spectrin (Chemicon). Reduced binding of anti-α spectrin to αSpIIΣ* in FA-A extracts was restored to normal in corrected FA-A cells (Fig. 4, *second panel*, *lanes 1–3*). Topoisomerase II was used as an internal control.

To determine whether αSpIIΣ* interacts with the FANCA and FANCC proteins, immunoprecipitation studies were carried out. Anti-FANCA immunoprecipitation and immunoblotting with either anti-αSpIIΣ* or anti-α spectrin demonstrated that αSpIIΣ* co-immunoprecipitated with FANCA and FANCC from HeLa chromatin-associated protein extracts (Fig. 5, *lane 1*). Preliminary studies indicate that FANCG also immunoprecipitated with FANCA and FANCC. None of these proteins co-immunoprecipitated from FA-A extracts, which lack FANCA and are deficient in αSpIIΣ* (Fig. 5, *lane 2*). In extracts from corrected FA-A cells, αSpIIΣ* again co-immunoprecipitated with FANCA and FANCC (Fig. 5, *lane 3*) and also FANCG (preliminary data). These results thus show that αSpIIΣ* forms a nuclear complex with these proteins. Faint bands were detected in the pre-immune precipitations (Fig. 5, *lane 4*) indicating a slight reactivity of the pre-immune sera with spectrin.

The binding of FANCA and FANCC to αSpIIΣ* was confirmed by anti-α spectrin immunoprecipitation (Fig. 6*A*, *lane 1*). Anti-α spectrin was used, even though it may be against a different isoform than our αSpIIΣ*, because our anti-αSpIIΣ* is of the IgM class and cannot be used effectively in immunoprecipitations. In FA-A extracts, no FANCA and reduced amounts of αSpIIΣ* and FANCC immunoprecipitated (Fig. 6*A*, *lane 2*). This corresponds to no expressed FANCA (Fig. 6*B*, *lane 2*) and reduced levels of αSpIIΣ* (Fig. 4, *top panel*, *lane 2*) and FANCC (Fig. 6*B*, *lane 2*) in FA-A nuclei. Reduced levels of FANCC in FA-A cell nuclei have also been reported by Ya-

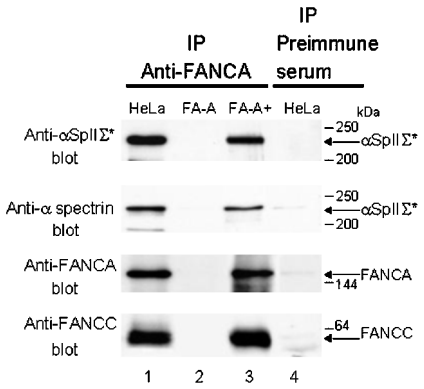

FIG. 5. **Binding of αSpIIΣ* to the FANCA and FANCC proteins in the nucleus.** Chromatin-associated proteins from HeLa (*lanes 1* and *4*), FA-A (HSC 72) (*lane 2*), and FA-A transduced (HSC 72–17) cells were immunoprecipitated with anti-FANCA (*lanes 1–3*) or pre-immune antiserum (*lane 4*) and immunoblotted with either anti-αSpIIΣ* (*top panel*), anti-α spectrin (*second panel*), anti-FANCA (*third panel*), or anti-FANCC (*fourth panel*).

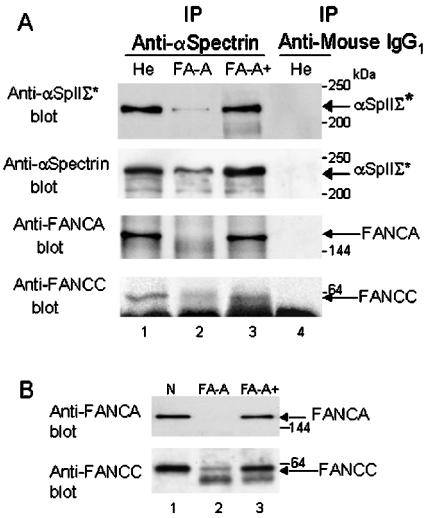

FIG. 6. **Confirmation of the binding of αSpIIΣ* to the FANCA and FANCC proteins by anti-α spectrin immunoprecipitation.** *A*, chromatin-associated proteins form HeLa (*lanes 1* and *4*), FA-A (HSC 72) (*lane 2*), and FA-A transduced (HSC 72–17) cells were immunoprecipitated with anti-FANCA (*lanes 1–3*) or anti-mouse IgG_1 (which served as a control) (*lane 4*) and immunoblotted with either anti-αSpIIΣ* (*top panel*), anti-α spectrin (*second panel*), anti-FANCA (*third panel*), or anti-FANCC (*fourth panel*). *B*, Western blot analysis of binding of anti-FANCA (*top panel*) and anti-FANCC (*bottom panel*) to proteins in chromatin-associated protein extracts from Hela (*lane 1*), FA-A (HSC 72) (*lane 2*), and FA-A transduced (HSC 72–17) cells.

mashita *et al.* (36). In extracts from corrected FA-A cells, αSpIIΣ*, FANCA, and FANCC again co-immunoprecipitated (Fig. 6*A*, *lane 3*). These studies confirm that FANCA and FANCC form a complex with αSpIIΣ* in the nucleus and that normal levels of αSpIIΣ* and FANCA in the nucleus are important for complex formation.

DISCUSSION

αSpIIΣ* has now been identified as associated with a protein complex that we have isolated from normal human cells and shown to be involved in repair of DNA interstrand cross-links (12–15). The exact isoform of this protein is not yet know and is currently being examined. Isoforms of each spectrin gene, generated by alternative pre-mRNA splicing, have been shown to occur (17, 18, 30). The presence of different isoforms in different cells and even in the same cell suggests distinct functions for each (18, 30, 31). Anti-αSpIIΣ* like anti-α spectrin had cross-reactivity with β spectrin. This probably indicates a common epitope that is being recognized by these antibodies. This is not unusual, because genes for nonerythroid α and β spectrin encode proteins that are approximately 58–60% identical to their erythroid counterparts (18, 28, 30, 32), and between nonerythroid and erythroid α and β spectrin there is also significant homology (18, 33).

The reduced levels of αSpIIΣ* that we observe in FA-A, FA-B, FA-C, and FA-D cells correlate with the decreased levels of DNA repair synthesis (*i.e.* unscheduled DNA synthesis) that are observed in these cells in response to DNA interstrand cross-linking agents and with the reduced ability of the protein complex to incise cross-linked DNA (13, 16). These values are approximately 25–35% of normal cells (16). Because we have shown that this protein complex contains a number of the proteins involved in nucleotide excision repair (*i.e.* XPR, RPA, TFIIH, HHR23B, XPG, ERCC1, XPF, and PCNA) (16), it is possible that a role for αSpIIΣ* in the repair process is to act as a scaffold to align these proteins so as to enhance their interactions. This alignment could be particularly important in repair of interstrand cross-links where recombination may be involved (34, 35). Reduced levels of αSpIIΣ* in the nucleus would thus be expected to reduce the efficiency of the repair process rather than inhibit it altogether, consistent with our experimental findings.

The FANCA and FANCC proteins have been shown to form a complex in the nucleus (36, 37). The present results show that αSpIIΣ* also forms a complex in the nucleus with these proteins. Because studies suggest that FANCA and FANCC may not bind directly to each other in the nucleus but that their interaction may involve another, as yet unknown, protein (36), it is possible that αSpIIΣ* is this protein. These finding suggest that αSpIIΣ* may act as a scaffold to align or enhance interactions between these two FA proteins, possibly other FA proteins such as FANCG, and proteins involved in DNA repair as well as in other cellular processes. In addition, because levels of αSpIIΣ* are reduced in cells from at least four FA complementation groups (FA-A, FA-B, FA-C, and FA-D), this suggests that these FA genes may all be involved in the stability or expression of this protein. This is further demonstrated by the finding that in FA-A cells expressing the *FANCA* cDNA, this deficiency in αSpIIΣ* is corrected as is the DNA repair defect (16).

The role of nonerythroid spectrin in the nucleus is not completely understood. Nonerythroid α-spectrin has been shown to be associated with the nuclear matrix (38). A number of studies suggest that the nuclear matrix is important in DNA repair (39–41). αSpIIΣ* may link the repair process to the nuclear matrix. α spectrin has also been shown to interact with a number of proteins (17, 18) and potentially some of these interactions could occur in the nucleus. For example, α spectrin contains a calmodulin binding site and has been shown to associate with this protein (19–21) and it also has a SH3 domain, which interacts with tyrosine kinases (17, 20, 22). Collectively these proteins, which potentially could bind to αSpIIΣ* in the nucleus, have been shown to interact with proteins involved in DNA synthesis, cell cycle progression, mitosis, gene expression, and signal transduction (17, 19–22, 42). α spectrin has also been shown to be involved in cell growth and differentiation (17, 18). A deficiency in αSpIIΣ* in FA cells could thus have far reaching consequences due to the number of systems affected. This could possibly explain some of the diverse cellular and clinical defects reported in FA, such as cell cycle defects, aberrant induction of apoptosis, and developmental abnormalities (1–3). Identification of other FA genes and their products should help determine how many of the FA proteins form a complex with αSpIIΣ* and whether these proteins also play a role in the stability or expression of αSpIIΣ*.

Elucidation of this relationship may help delineate the function of these FA proteins in the nucleus, the nature of their interaction with αSpIIΣ*, and their role in the deficiency in αSpIIΣ* observed in FA cells.

Acknowledgments—We thank Robert Lockwood for culturing of the human cell lines and Dr. W. Clark Lambert for critically reviewing the manuscript.

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