Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 6712–6716, July 1994
Medical Sciences

The Fanconi anemia polypeptide FACC is localized to the cytoplasm

(mitomycin-C/leukemia susceptibility)

TAKAYUKI YAMASHITA, DWAYNE L. BARBER, YUAN ZHU, NAN WU, AND ALAN D. D'ANDREA*

Division of Pediatric Oncology and Division of Cellular and Molecular Biology, Dana–Farber Cancer Institute, Harvard Medical School, Boston, MA 02115

Communicated by Louis M. Kunkel, March 29, 1994 (received for review January 3, 1994)

ABSTRACT **Fanconi anemia (FA) is an autosomal recessive disease characterized by congenital anomalies, aplastic anemia, and chromosomal instability. A cDNA encoding the FA complementation group C (FACC) polypeptide was recently cloned [Strathdee, C. A., Gavish, H., Shannon, W. R. & Buchwald, M. (1992) *Nature (London)* 356, 763–767]. To further characterize this polypeptide, we generated a rabbit polyclonal antiserum against its carboxyl terminus. We used this antiserum to analyze the FACC polypeptide from normal or mutant (FA) lymphoblast cell lines. By immunoprecipitation, the wild-type FACC was a 60-kDa protein, consistent with its predicted molecular mass. FA group C cell lines expressed full-length FACC, truncated FACC, or no detectable FACC polypeptide. In addition, the antiserum specifically immunoprecipitated a 50-kDa and a 150-kDa FACC-related protein (FRP-50 and FRP-150). Unexpectedly, cell fractionation and immunofluorescence studies demonstrated that the FACC polypeptide localizes to the cytoplasm. In conclusion, we have generated an antiserum specific for the human FACC polypeptide. The antiserum should be useful for screening FA cells for mutant FACC polypeptides and for identifying and cloning FACC-related proteins.**

Fanconi anemia (FA) is an autosomal recessive disease characterized by progressive pancytopenia, cancer susceptibility, multiple congenital abnormalities, and chromosomal instability (1, 2). Cells from FA patients are hypersensitive to DNA cross-linking agents, such as mitomycin-C (MMC) and diepoxybutane (3). Several studies have suggested that the molecular defect of FA is one of DNA repair (4, 5). Cell fusion studies have identified at least four complementation groups (6). Recently, a cDNA for FA complementation group C (FACC) was cloned (7, 8). The primary sequence of the predicted 558-amino acid FACC polypeptide has no homology with known proteins, and its function remains unknown.

Mutation analysis of the FACC gene indicates that approximately 15% of FA patients are group C [referred to here as FA(C)] (9, 10). Several types of mutations have been found within the FACC gene (9) although the mutations do not cluster within any specific region of the gene. The most frequent mutations are the IVS4+4 A-to-T mutation, a splice mutation that results in loss of exon 4 (10), and the delG322 mutation, which results in a frameshift and a premature stop codon in exon 1 (7, 9).

To help elucidate the function of the FACC polypeptide, we have raised a rabbit polyclonal antibody against the carboxyl terminus of FACC. The anti-FACC antibody recognized wild-type FACC in normal lymphoblasts and mutant forms of FACC in lymphoblasts derived from FA(C) patients. The antiserum also immunoprecipitated two FACC-related proteins. By cell fractionation and immunofluorescence studies, the wild-type FACC polypeptide localizes to the cytoplasm. FACC is therefore unlikely to play a direct role in the repair of DNA damage.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Cell Lines and Culture Conditions. The Epstein–Barr virus (EBV)-transformed normal and mutant (FA) lymphoblast lines have been previously described (7, 10). Human HSC536N cells were generously supplied by M. Buchwald (7). PD4, PD7, and PD153 cells were obtained from M. Grompe and the Fanconi Anemia Cell Repository (Oregon Health Sciences University) (10). 4510C cells were obtained from the ATCC. All lymphoblast lines were maintained as suspension cultures in RPMI medium 1640 supplemented with 15% heat-inactivated fetal calf serum (growth medium). They were grown at 37°C in a humidified, 5% CO_2-containing atmosphere.

Development of an Anti-FACC Antiserum. To generate a polyclonal antibody against the FACC polypeptide, a glutathione *S*-transferase (GST)-FACC fusion protein containing the carboxyl terminus of FACC (amino acids 281–558) was expressed in *Escherichia coli* and purified. Purification of the GST fusion protein was performed as described previously (11) with a few modifications. The bacteria were suspended in a lysis buffer containing lysozyme (1 mg/ml), dithiothreitol (5 mM), *N*-lauroylsarcosine (1.5%) (12). Triton X-100 (1%), and protease inhibitors (aprotinin at 2 μg/ml, leupeptin at 10 μg/ml, pepstatin at 1 μg/ml, and 1 mM phenylmethylsulfonyl fluoride), sonicated, and then centrifuged at 12,000 × *g*. From the supernatant, the GST-FACC fusion protein was purified by using glutathione-Sepharose (Pharmacia). The GST-FACC fusion protein was injected into rabbits (100 μg per injection) by standard techniques (13). Antiserum was prepared by depletion of anti-GST immunoglobulin and by purification with staphylococcal protein A-Sepharose.

Metabolic Labeling and Immunoprecipitation. Metabolic labeling of EBV-transformed lymphoblasts with [^{35}S]methionine (2 hr) and immunoprecipitation of labeled proteins were performed as described previously (14). Briefly, cell extracts in TBS (50 mM Tris-buffered isotonic saline, pH = 7.5) containing 5 mM EDTA, 1% (vol/vol) Triton X-100, 0.2% sodium dodecyl sulfate (SDS), and sodium deoxycholate at 5 mg/ml were precleared two times with preimmune serum (1:100) and subsequently mixed with the rabbit antiserum against FACC (1:200) or a monoclonal antibody against p53 (Oncogene Science, Ab-2) (1:100). Alternatively, where indicated, a monoclonal antibody against the influenza virus hemagglutinin (HA) (monoclonal antibody 12CA5) was used to immunoprecipitate the epitope-tagged FACC polypeptide

Abbreviations: FA, Fanconi anemia; MMC, mitomycin-C; FACC, FA complementation group C; FA(C), FA group C; EBV, Epstein–Barr virus; GST, glutathione *S*-transferase; HA, influenza virus hemagglutinin; FRP, FACC-related protein.

*To whom reprint requests should be addressed at: Dana–Farber Cancer Institute, Pediatric Oncology, 44 Binney Street, Boston, MA 02115.

(1:100). Protein A-Sepharose (Pharmacia) immune complexes were washed three times with TBS containing 1% Triton X-100 and 0.1% SDS, and subjected to SDS/polyacrylamide gel electrophoresis (PAGE) on 10% or 6–12% gradient gels as indicated.

Cell Fractionation. ^{35}S-labeled lymphoblasts were washed three times in ice-cold phosphate-buffered saline and swollen for 15 min in ice-cold hypotonic buffer containing 10 mM Hepes (pH 7.4), 2 mM $MgCl_2$, 40 mM KCl, and protease inhibitors (as above), and ruptured in a Dounce homogenizer. After 20 strokes, phase-contrast microscopy indicated that more than 95% of the cells were disrupted. The homogenates were centrifugated at 200 × *g* for 15 min. The pellet was used as a nuclear fraction. The supernatant was further subjected to centrifugation at 300,000 × *g* for 10 min. The pellet (membrane fraction) contained lysosomal, mitochondrial, and plasma membrane proteins. The supernatant was used as a soluble, cytoplasmic fraction. Each fraction was subjected to immunoprecipitation with the anti-FACC antibody or the anti-p53 monoclonal antibody.

Expression of an Influenza Hemagglutinin (HA) Epitope-Tagged FACC in Lymphoblasts. The wild-type FACC polypeptide and the mutant (L554P) FACC polypeptide were tagged with the HA epitope (YPYDVPDYA) at its amino terminus by standard methods (15). Polymerase chain reaction using a 5′ primer (AAAGGTACCGCTTTTTCCAAGATGTACCCATACGATGTTCCAGATTACGCTCAAGATTCAGTAGATCTT) and a 3′ primer (AAACTCGAGCTAGACTTGAGTTCGCAGCTCTTTAAGGAG) or (AAACTCGAGCTAGACTTGAGTTCGCGGCTCTTTAAGGAG) were used to amplify from pREP-FACC as the cDNA template. The amplified cDNA products were inserted into the *Kpn* I and *Xho* I sites of the pREP4 vector (Stratagene). pREP-HA-FACC or pREP-HA-FACC(L554P) (20 μg) was electroporated into HSC536N cells (10^7 cells), as previously described (14). Selection with hygromycin (0.1 mg/ml) in RPMI medium 1640 was initiated 48 hr after electroporation. Selected cells were analyzed for the presence of HA-FACC (or mutant) polypeptide by immunoprecipitation.

MMC Sensitivity Assay. Lymphoblasts (10^4 cells) were cultured for 4 days in growth medium (200 μl) containing the indicated concentrations of MMC. The number of viable cells was then measured by the XTT [3′-1-phenylaminocarbonyl-3,4-tetrazolium-bis(4-methoxy-6-nitrobenzenesulfonic acid)] assay (16).

Immunofluorescence. A suspension of cells that had grown to confluence (60 μl) was air-dried on the surface of a coverslip and fixed in methanol (−20°C) for 2 min. The coverslip was washed in phosphate-buffered saline and incubated with phosphate-buffered saline containing bovine serum albumin (BSA; 2 mg/ml) at 37°C for 1 hr. After a brief wash, monoclonal (anti-HA) antibody (12CA5) (1:100 dilution in phosphate-buffered saline/3% BSA) (13) was applied to the coverslip and incubated at 37°C for 1 hr. The coverslip was washed in phosphate-buffered saline plus BSA (2 mg/ml) and then phosphate-buffered saline alone and incubated with Texas red-conjugated goat anti-mouse IgG secondary antibody (Jackson ImmunoResearch) (1:1000 dilution in phosphate-buffered saline/3% BSA) at 37°C for another hour. The coverslip was washed, mounted on a slide, and photographed.

RESULTS

Identification of Wild-Type and Mutant FACC Polypeptides in Lymphoblast Lines. The FACC genotypes of the EBV-transformed (FA) lymphoblast lines used for the experiments are shown in Table 1. Predicted polypeptides for the wild-type gene and each mutant are shown schematically in Fig. 1. HSC536N cells are compound heterozygotes; one allele contains a missense mutation [T to C (1913)], predicting an L554P (Leu-554 → Pro) change in FACC, and the other allele is deleted and therefore not expressed (7). PD153 and 4510C cells are homozygous for the IVS4+4 A-to-T mutation, predicting a protein with an in-frame deletion of 38 amino acids, normally encoded by exon 4 (10). PD4 cells are compound heterozygotes (9); one allele has the delG322 mutation, predicting a frameshift and a truncated protein of 44 amino acids (7), and the other allele has a nonsense mutation in exon 6 [C to T (808)], predicting a truncated protein of 185 amino acids (17). PD7 cells are lymphoblasts from a normal adult control. We initially raised a rabbit polyclonal anti-FACC antiserum against a GST fusion protein containing the carboxyl terminus of wild-type FACC (amino acids 281–558).

Table 1. Genotype of FA(C) lymphoblast lines

Cell line	FACC genotype*	
	Allele 1	Allele 2
HSC536N	T to C (1913)	Deleted
PD153	IVS4+4 A to T	IVS4+4 A to T
4510C	IVS4+4 A to T	IVS4+4 A to T
PD4	delG (322)	C to T (808)

IVS4+4 A to T indicates a single base change in the 4th intronic base, changing the sequence from A to T.
*Base pair position shown in parentheses.

To identify the wild-type or mutant FACC polypeptides, the indicated cell lines were metabolically labeled with [^{35}S]methionine, and radiolabeled proteins were immunoprecipitated with the anti-FACC antiserum (Fig. 2). The wild-type FACC polypeptide (60 kDa) was specifically immunoprecipitated from PD7 lysates with the immune serum (Fig. 2*A*, lane 10) but not with the preimmune serum (lane 9). The immune serum also recognizes FACC as a 60-kDa polypeptide by immunoblot (data not shown). The full-length mutant FACC protein (L554P) was present at a similar level in lysates from HSC536N cells (lane 1), indicating that the mutant protein was stably expressed in these cells. PD153 and 4510C cells have a truncated form of the FACC polypeptide (55 kDa) (lanes 3 and 5). This truncated protein is consistent in size with an in-frame deletion of 38 amino acids, resulting from aberrant mRNA splicing and loss of exon 4 (10). No FACC polypeptide was observed in PD4 cell lysates (lane 7). These cells may contain the truncated 44- and 185-amino acid polypeptides, shown schematically in Fig. 1; however, the anti-FACC antiserum would not recognize these forms of FACC.

Identification of FACC-Related Proteins. A 50-kDa protein (called FRP-50, for FACC-related protein) immunoprecipitated with the anti-FACC antibody from normal cell (PD7) lysates (Fig. 2*A*, lane 10) but not with the preimmune serum (lane 9). FRP-50 was detected in PD7 and HSC536N (lane 1), but not in PD153 or 4510C cells (lanes 3 and 5, respectively). FRP-50 is unlikely to be a proteolytic fragment of FACC or a protein associated with FACC, since it is present in PD4 cells (lane 7), which contain no full-length FACC polypeptide.

In addition, the anti-FACC antiserum specifically recognized a 150-kDa protein (FRP-150) in PD7 cells, demonstrated when immune complexes were analyzed on a 6–12% gradient polyacrylamide gel (Fig. 2*B*, lane 4). FRP-150 was also found in lysates from PD4 (Fig. 2*B*, lane 2) and from PD153 and 4510C cells (data not shown). FRP-150, like FRP-50, is unlikely to be an FACC-associated protein, because it is present in PD4 cells, which lack full-length FACC polypeptide. FACC, FRP-50, and FRP-150 were also expressed in erythroleukemia cell lines and in several EBV-

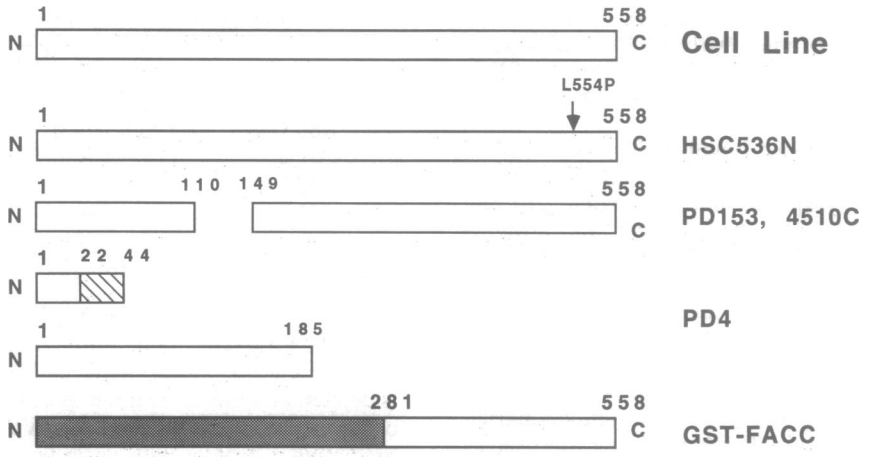

FIG. 1. Mutations of the FACC polypeptide found in FA(C) patients. The wild-type FACC polypeptide (558 amino acids) is shown schematically at the top, with amino (N) and carboxyl (C) termini labeled. The indicated FA(C) cell lines (shown at the right) are predicted from their genotypes (Table 1) to contain the FACC mutant polypeptides shown. The T to C (1913) allele encodes the L554P mutant. The IVS4+4 A-to-T allele encodes an FACC mutant lacking the 37 amino acids encoded by exon 4. PD4 cells are predicted from their genotype to contain two truncated FACC polypeptides, one of 44 amino acids and one of 185 amino acids. A GST fusion protein, containing FACC amino acid sequence 281–558, was used to generate an anti-FACC antiserum.

transformed lymphoblast lines from patients with non-C FA (data not shown).

The FACC Polypeptide Is Localized to the Cytoplasm. To determine which cellular compartment contained FACC, subcellular fractionation was performed on ^{35}S-labeled normal lymphoblasts. Immunoprecipitation of FACC from the cellular fractions (Fig. 3, lanes 1–4) demonstrated that FACC (and FRP-50) were present in the soluble cytosolic fraction (lane 3). The FACC band intensity was equal to the band intensity for the normalized whole cell lysate (lane 1), indicating that all FACC was in the soluble cytosol. No FACC polypeptide was found in the nuclear fraction (lane 2) or in the membrane fraction (lane 4). Repeat analysis (data not shown) also demonstrated the presence of FRP-150 in the cytoplasmic fraction. As an internal control, we used an anti-p53 monoclonal antibody to immunoprecipitate ^{35}S-labeled p53 from the fractions (Fig. 3, lanes 5–8). Consistent with previous reports (18, 19), p53 was found primarily in the nuclear fraction under these conditions (Fig. 3, lane 6).

Since the anti-FACC antiserum is immunoreactive with several cellular proteins, including FACC, FRP-50, and FRP-150, it is not suitable for specifically identifying FACC by immunofluorescence. We therefore generated an epitope-tagged FACC polypeptide (Fig. 4). The 9-amino acid epitope

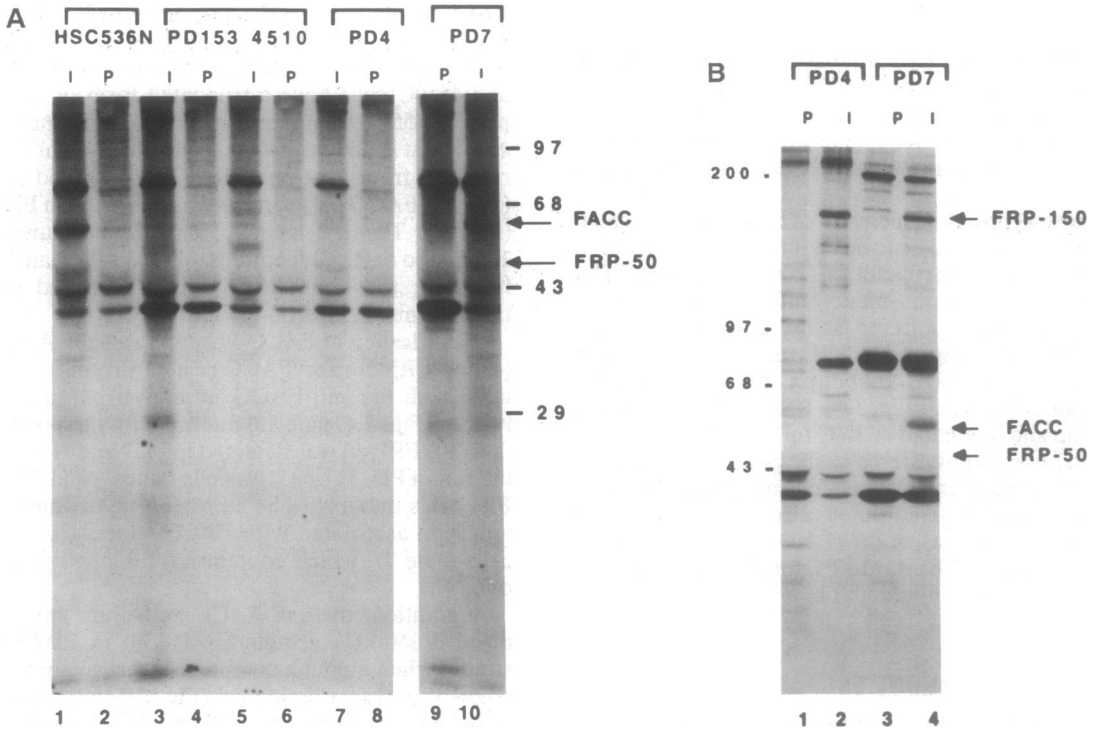

FIG. 2. The anti-FACC antibody recognizes wild-type FACC, mutant FACC polypeptides, and FACC-related proteins. (*A*) The indicated EBV-transformed lymphoblast lines were metabolically labeled with [^{35}S]methionine, and radiolabeled proteins were immunoprecipitated with the anti-FACC antiserum. I, immune serum (lanes 1, 3, 5, 7, 10); P, preimmune serum (lanes 2, 4, 6, 8, 9). Control (normal) cells (PD7) are shown in lanes 9 and 10. Labeled proteins were resolved by 10% PAGE. Numbers on the right are molecular mass (kDa). FRP-50, 50-kDa FACC-related protein. (*B*) As in *A*, except labeled proteins were resolved by gradient (6–12%) PAGE. FRP-150, 150-kDa FACC-related protein.

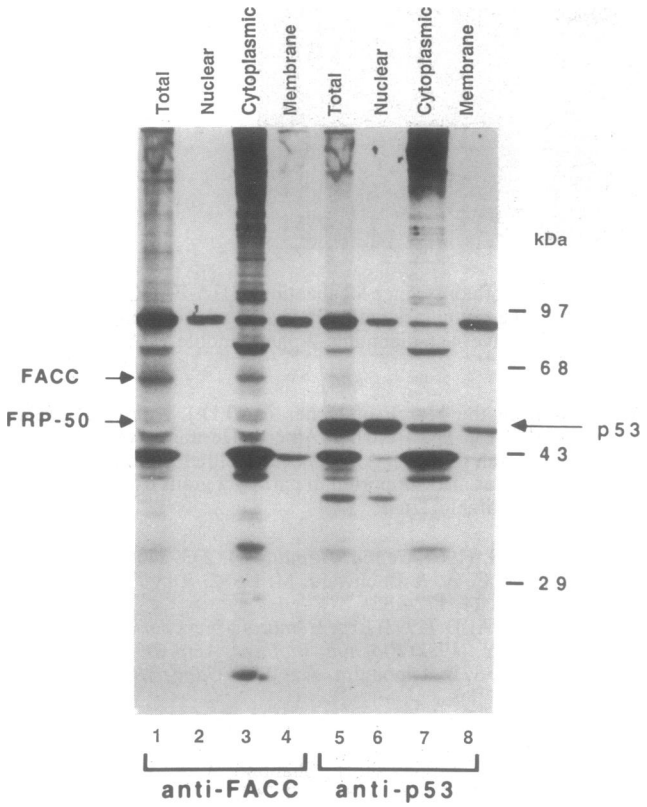

FIG. 3. Localization of the endogenous FACC polypeptide to the cytoplasm. Metabolically labeled normal EBV-transformed lymphoblasts (PD7) were homogenized and fractionated as described in the text. ^{35}S-labeled proteins from the indicated fractions were immunoprecipitated with the anti-FACC antibody (lanes 1–4) or with a monoclonal antibody to p53 (lanes 5–8).

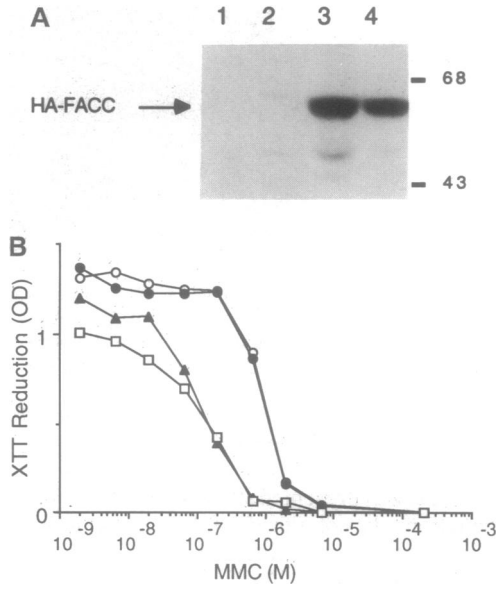

FIG. 4. Complementation of the MMC-sensitive phenotype of FA(C) cells by the epitope (HA)-tagged FACC cDNA. (*A*) HSC536N cells were transfected with pREP4-mock (lane 2), pREP4-HA-FACC (lane 3), or pREP4-HA-FACC(L554P) (lane 4). Transfected, selected HSC536N cells (or normal lymphoblasts, lane 1) were metabolically labeled, and ^{35}S-labeled proteins were immunoprecipitated with 12CA5. (*B*) MMC sensitivity of HSC536N cells transfected with pREP4-mock (□), HA-FACC(L554P) (▲), or HA-FACC (○). As control, a normal lymphoblast line (●) is shown. MMC concentrations ranged from 2 nM to 300 μM. The EC_{50} (the drug concentration giving a 50% reduction in cell viability) was calculated and is indicated in the text. OD, optical density at 450 nm.

of the HA protein was placed at the amino terminus of either wild-type or mutant (L554P) FACC by standard techniques. The cDNAs encoding these tagged proteins were introduced into HSC536N cells by electroporation. The expression of HA-FACC or HA-FACC(L554P) mutant polypeptides was confirmed by immunoprecipitation with an anti-HA monoclonal antibody (15) (60-kDa protein, Fig. 4*A*, lanes 3 and 4, respectively). HA-FACC and HA-FACC(L554P) proteins were expressed at comparable levels, consistent with earlier experiments demonstrating the stable expression of the FACC(L554P) mutant (Fig. 2*A*, lane 1). Neither FRP-50 nor FRP-150 was observed in the immune complexes (data not shown), confirming that these latter proteins are related but not associated proteins of FACC.

We next assayed the transfected cell lines for their cellular sensitivity to MMC (Fig. 4*B*). Mock-transfected HSC536N cells were sensitive to MMC (EC_{50} = 105 nM), consistent with published studies (7). HSC536N cells, expressing HA-FACC, demonstrated enhanced cellular resistance to MMC (EC_{50} = 900 nM), similar to normal lymphoblasts. This result demonstrates that the presence of the HA-epitope tag at the amino terminus of the FACC polypeptide does not alter its normal function in the cell. In contrast, HSC536N cells transfected with HA-FACC(L554P) remained sensitive to MMC.

To verify the cytoplasmic localization of FACC, we used immunofluorescence localization of the wild-type HA-FACC polypeptide expressed in the complemented HSC536N lymphoblasts (Fig. 5). Transfected HSC536N cells were stained with the anti-HA monoclonal antibody followed by a Texas red-conjugated anti-mouse secondary antibody. The exogenous HA-FACC polypeptide demonstrated cytoplasmic (perinuclear) localization (Fig. 5*A*). Counterstaining with the DNA-specific dye 4′,6-diamidino-2-phenylindole delineated the nuclei of these same cells (Fig. 5*B*). Transfected cells stained with secondary antibody alone showed no background staining (Fig. 5*C*).

DISCUSSION

We have raised a rabbit polyclonal antiserum against the carboxyl terminus of FACC. Using this antiserum, we have identified the wild-type FACC as a 60-kDa cytoplasmic protein. HSC536N cells express normal levels of a mutant protein (L554P), while FACC cell lines with a splice mutation (IVS4+4 A to T) have decreased levels of a 55-kDa truncated protein. The antibody recognized no full-length FACC polypeptide in PD4 cells. In addition, the antiserum specifically immunoprecipitated a 50-kDa and a 150-kDa FACC-related protein (FRP-50 and FRP-150, respectively). Excess GST-FACC polypeptide specifically competed not only with FACC but also with FRP-50 and FRP-150 (data not shown).

Our results demonstrate that the mutant protein (L554P) is stably expressed in HSC536N cells. HSC536N cells are highly sensitive to MMC; transfection with the wild-type FACC cDNA complements this phenotype (7), but transfection with the mutant (L554P) protein failed to correct this phenotype. These results, taken together, suggest that the carboxyl terminus of FACC contains at least one important functional domain. Secondary structure analysis of the carboxyl terminus of FACC predicts an α-helical region of 50 amino acids; the L554P mutation disrupts this α-helix (A. D., unpublished observation). On the other hand, PD153 and 4510C cells, which are homozygous for the splice mutation (IVS4 + 4 A to T), expressed a decreased level of a truncated (55-kDa) protein. This finding suggests that the 55-kDa truncated FACC polypeptide is either unstable or functionally inactive. This splice mutation is the most common abnormality in FACC

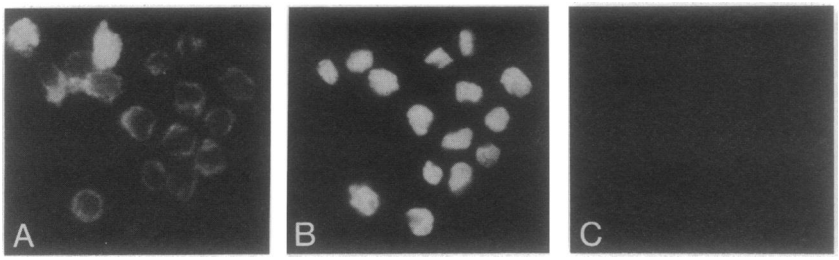

FIG. 5. Localization of epitope-tagged FACC polypeptide to the cytoplasm. (*A*) Immunofluorescence localization of HA-FACC in HSC536N cells. HSC536N cells, transfected with pREP4-HA-FACC, were stained with 12CA5. The secondary antibody was Texas red-conjugated goat anti-mouse antibody. (*B*) The same cells were counterstained with the DNA-specific dye 4',6-diamidino-2-phenylindole (DAPI). (*C*) The transfected cells were stained with the secondary antibody alone.

patients (9, 10), and the anti-FACC antiserum described here should therefore be useful for its screening.

The identities of FRP-50 and FRP-150 remain to be determined. FRP-150 was present in all cell lines tested, implying that FACC mutations do not alter FRP-150. Thus, it is likely that FRP-150 is a homologous protein encoded by a distinct gene. One intriguing possibility is that FRP-150 is encoded by a gene corresponding to a different complementation group of FA.

FRP-50, in contrast, was present in normal (PD7) and HSC536N cells but absent from PD153 or 4510C cells, which lack the full-length FACC. FRP-50 seems, therefore, to be derived from the FACC gene itself, perhaps as an alternative splice variant of FACC. Previous studies have demonstrated that even normal lymphoblasts express alternative splice variants of FACC, including a deletion of exon 13 (17, 20). Additionally, cells that are homozygous for an exon 6 nonsense mutation express a truncated mRNA, lacking exon 6 (17), demonstrating that exon skipping may occur. The presence of FRP-50 in PD4 cells could result from skipping of exon 6 and effective removal of the R185X nonsense mutation. Taken together, our results suggest that an alternatively spliced FACC mRNA could result in translation of FRP-50. To further support this idea, the anti-FACC antiserum also recognizes two forms of the FACC polypeptide in immune complexes from murine cells (data not shown). The murine FACC has an additional exon, encoding 33 amino acids, which may account for one of these proteins (21). The further identification and cloning of FRP-50 and FRP-150 may be useful in elucidating the function of FACC.

Our data demonstrate that the primary localization of FACC is the cell cytoplasm. We have not ruled out the possibility that FACC may be targeted to the nucleus under different stages of the cell cycle or after treatment with DNA-crosslinking agents. Further studies, including immunoelectron microscopy, will be required to test these possibilities.

The primary cytoplasmic localization of FACC has important implications for its intracellular function. FACC is unlikely to play a direct role in the repair of crosslinked DNA. Its role in DNA repair may be an indirect one, such as the cytoplasmic modification or activation of nucleases that can, in turn, effect DNA repair in the nucleus. Alternatively, FACC may play a role in the scavenging of oxygen radicals or in controlling cellular levels of NAD^+ (22, 23). Previous studies indicate that FA cells are hypersensitive to oxygen (24–26). Oxygen radical sensitivity could account for the enhanced sensitivity of FA cells to DNA crosslinking agents (27). A direct comparison of oxygen metabolism by FA cells and FA cells complemented with the FACC cDNA may further elucidate its function.

We thank Markus Grompe for helpful discussions and for providing PD4 and PD7 cell lines. We thank Manuel Buchwald for HSC536N cells and for the FACC cDNA. We thank Cristin Corless for technical assistance and Pam Silver, Tim Jinks, William Kaelin, and Gerald Fasman for helpful discussions. This work was supported by grants from the March of Dimes (A.D.D.), from the Uehara Foundation (T.Y.), and from the Alberta Heritage Foundation for Medical Research (D.L.B.) A.D.D. is a Lucille P. Markey Scholar, and this work was also supported in part by a grant from the Lucille P. Markey Charitable Trust.

1. Fanconi, G. (1967) *Semin. Hematol.* **4**, 233–240.
2. Strathdee, C. A. & Buchwald, M. (1992) *Am. J. Ped. Hematol./Oncol.* **14**, 177–185.
3. Auerbach, A. D. (1993) *Exp. Hematol. (New York)* **21**, 731–733.
4. Fujiwara, Y. (1982) *Biochim. Biophys. Acta* **699**, 217–225.
5. Sasaki, M. S. & Tonomura, A. (1973) *Cancer Res.* **33**, 1829–1836.
6. Strathdee, C. A., Duncan, A. M. V. & Buchwald, M. (1992) *Nature Genet.* **1**, 196–198.
7. Strathdee, C. A., Gavish, H., Shannon, W. R. & Buchwald, M. (1992) *Nature (London)* **356**, 763–767.
8. Strathdee, C. A., Gavish, H., Shannon, W. R. & Buchwald, M. (1992) *Nature (London)* **358**, 434.
9. Verlander, P. C., Lin, J. D., Udono, M. U., Zhang, Q., Gibson, R. A., Mathew, C. G. & Auerbach, A. D. (1994) *Am. J. Hum. Genet.* **54**, 595–601.
10. Whitney, M. A., Saito, H., Jakobs, P. M., Gibson, R. A., Moses, R. E. & Grompe, M. (1993) *Nature Genet.* **4**, 202–205.
11. Kaelin, W. G., Pallas, D. C., DeCaprio, J. A., Kaye, F. J. & Livingston, D. M. (1991) *Cell* **64**, 521–532.
12. Frangioni, J. V. & Neel, B. G. (1992) *Anal. Biochem.* **210**, 179–187.
13. Harlow, E. & Lane, D. (1988) *Antibodies: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 92–121.
14. D'Andrea, A. D., Yoshimura, A., Youssoufian, H., Zon., L. I., Koo, J.-W. & Lodish, H. F. (1991) *Mol. Cell. Biol.* **11**, 1980–1987.
15. Egan, S. E., Giddings, B. W., Brooks, M. W., Buday, L., Sizeland, A. M. & Weinberg, R. A. (1993) *Nature (London)* **363**, 45–51.
16. Roehm, N. W., Rodgers, G. H., Hatfield, S. M. & Glasebrook, A. L. (1991) *J. Immunol. Methods* **142**, 257–265.
17. Gibson, R., Hajianpour, A., Murer-Orlando, M., Buchwald, M. & Mathew, C. G. (1993) *Hum. Mol. Genet.* **2**, 797–799.
18. Pinhasi-Kimhi, O., Michalovitz, D., Ben-Zeev, A. & Oren, M. (1986) *Nature (London)* **320**, 182–185.
19. Momand, J., Zambetti, G. P., Olson, D. C., George, D. & Levine, A. J. (1992) *Cell* **69**, 1237–1245.
20. Gibson, R. A., Buchwald, M., Roberts, R. G. & Mathew, C. G. (1993) *Hum. Mol. Genet.* **2**, 35–38.
21. Wevrick, R., Clarke, C. A. & Buchwald, M. (1993) *Hum. Mol. Genet.* **2**, 655–662.
22. Berger, N. A., Berger, S. J. & Catino, D. M. (1982) *Nature (London)* **299**, 271–273.
23. Klocker, H., Auer, B., Hirsch-Kauffmann, M., Altmann, H., Burtscher, H. J. & Schweiger, M. (1983) *EMBO J.* **2**, 303–307.
24. Gille, J. J. P., Wortelboer, H. M. & Joenje, H. (1987) *Hum. Genet.* **77**, 28–31.
25. Schindler, D. & Hoehn, H. (1988) *Am. J. Hum. Genet.* **43**, 429–435.
26. Dallapiccola, B., Porfirio, B., Mokini, V., Alimena, G., Isacchi, G. & Gandini, E. (1985) *Hum. Genet.* **69**, 62–65.
27. Pritsos, C. A. & Sartorelli, A. C. (1986) *Cancer Res.* **46**, 3528–3532.