UBE2T, the Fanconi Anemia Core Complex, and FANCD2 Are Recruited Independently to Chromatin: a Basis for the Regulation of FANCD2 Monoubiquitination

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Received 22 March 2007/Returned for modification 10 September 2007/Accepted 3 October 2007

The Fanconi anemia (FA) nuclear core complex and the E2 ubiquitin-conjugating enzyme UBE2T are required for the S phase and DNA damage-restricted monoubiquitination of FANCD2. This constitutes a key step in the FA tumor suppressor pathway, and much attention has been focused on the regulation at this point. Here, we address the importance of the assembly of the FA core complex and the subcellular localization of UBE2T in the regulation of FANCD2 monoubiquitination. We establish three points. First, the stable assembly of the FA core complex can be dissociated of its ability to function as an E3 ubiquitin ligase. Second, the actual E3 ligase activity is not determined by the assembly of the FA core complex but rather by its DNA damage-induced localization to chromatin. Finally, UBE2T and FANCD2 access this subcellular fraction independently of the FA core complex. FANCD2 monoubiquitination is therefore not regulated by multiprotein complex assembly but by the formation of an active E2/E3 holoenzyme on chromatin.

Patients with the rare genetic disorder Fanconi anemia (FA) have a common defect in a DNA damage response pathway that contributes to the maintenance of genome stability (12). The FA pathway consists of a high-molecular-weight nuclear core complex which contains at least 10 subunits (FANCA, -B, -C, -E, -F, -G, -L, and -M, FAAP100, and FAAP24 proteins, known as the FA core complex) (20, 22, 27), as well as five additional proteins, FANCD2 (8, 38), FANCI (6, 35, 36), FANCD1 (BRCA2) (11), FANCN (PALB2) (33, 34), and FANCJ (BRIP1) (3, 16). DNA replication and DNA damage somehow activate the FA core complex to monoubiquitinate the FANCD2/FANCI heterodimer (9, 35, 36). The FA core complex and ubiquitinated FANCD2/FANCI are then thought to process DNA lesions. However, we still do not know how this FA core complex is activated and how it functions directly within such a DNA damage response pathway.

Most current studies that have determined interactions between FA core complex subunits suggest the existence of distinct subcomplexes in the cytosolic and nuclear compartments (5, 19, 32). Indeed, the stability of the FA core complex is severely compromised in most FA patients, and this is due to mutational inactivation of any of the FA core complex genes (8). In addition, the nuclear import of some of the FA core complex components is regulated and their localization is interdependent (15, 29). Cumulatively, all these observations have led to a compelling proposal that the FA core complex is sequentially assembled and that this process may regulate its activity. The FA core complex E3 ligase activity is tightly restricted to S and G2 phases of the cell cycle and can be further triggered by DNA damage (23, 25, 37). One way to ensure this restriction is to control the assembly of the FA core complex in response to such signals. In addition to complex assembly, some of the core complex components (such as FANCM, FANCE, and FANCG) are phosphorylated in response to DNA replication and damage (21, 24, 26, 31, 39). Such modifications may also be important for regulation.

The key function of the FA core complex is to stimulate the site-specific monoubiquitination of the FANCD2 and FANCI proteins (8, 36). Hence, the FA core complex likely functions as a multisubunit E3 ubiquitin ligase. Support for this comes from the fact that at least one intrinsic component of the FA core complex, FANCE, can bind and perhaps recruit FANCD2 for modification (29). More importantly, another essential component of the complex, FANCL, has a PHD/RING finger domain (20). This domain of FANCL binds to the E2-conjugating enzyme, UBE2T, and stimulates its autoubiquitination (18, 20). In addition, UBE2T has been shown to be necessary for the efficient DNA damage-induced monoubiquitination of FANCD2 (18). Thus, the FA core complex most likely assembles with UBE2T as an active E2/E3 holoenzyme. However, how and where such interactions occur and whether they contribute to regulation remain to be determined.

This study investigates the relevance of an assembly of the FA core complex as well as its subcellular localization with UBE2T to its E3 ubiquitin ligase activity. Using the model vertebrate genetic system DT40, we consolidate previous work and show that the E3 ligase activity of the FA core complex can be dissociated from its assembly. By creating novel DT40 strains, we also establish that the FA core complex is constitutively assembled and therefore stable throughout the cell.

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1 Published ahead of print on 15 October 2007.
cycle. Molecular size and abundance of the FA core complex are hardly affected, although the complex accumulates on chromatin in response to DNA replication and damage. Interestingly, UBE2T is constitutively present in the chromatin fraction, while the FA core complex and its substrate FANCD2 accumulate there independently in a restricted manner. Cumulatively, these observations allow us to put forward a model that may explain how the FA pathway is activated in response to DNA replication and DNA damage.

MATERIALS AND METHODS

Isolation of chicken FANCL cDNA and plasmid construction. Full-length cDNA of chicken FANCL was derived from a screen of a chicken embryonic fibroblast cDNA library (gift from A. Neito and D. Wilkinson). FANCL cDNA with adapted XhoI/NotI restriction sites was amplified by PCR and ligated into pBluescript. N-terminal Tag-tap cDNA (encoding two protein A domains, a tobacco etch virus protease cleavage site, and a calmodulin binding domain) with flanking XhoI/Sall sites was cloned into the XhoI site of pBluescript-FANCL (Sall site destroyed). Subsequently, XhoI/NotI sites of the TAP-FANCL fragment were filled in with Klenow enzyme and ligated into the Sma site of pExpress (gift from Jean-Marie Buerstedde). The expression cassette of TAP-FANCL (with a beta-actin promoter) from pExpress was then ligated into pLox-Bsr (gift from Jean-Marie Buerstedde). Stable transfections of DT40 strains with TAP-FANCL were performed as described previously (28).

Cysteine point mutations, Cys305 to Ala and Cys357 to Ala, in TAP-FANCL were generated by site-directed mutagenesis (QuiikChange II XL site-directed mutagenesis kit; Stratagen) using the following primer sets: AGTGACTGA CTAA GGACCG TGGATCTGC T GATC GCCGCT AT and TGAAGGCGG TAGCA GATCATGCTT AG TAAAGGCT ACT for Cys305Ala; ATCTTTGTTG ATTGTTCTA CTGCAACAG ACTGACGAC TG and CAGTCTTCT GGTCTGTTG GAAATGGGACA TTCCACAAG AT for Cys357Ala.

DT40 cell culture and transfections. DT40 cells were cultured at 37°C in RPMI 1640 supplemented with 7% fetal calf serum (Gibco-BRL). 3% chicken serum (Gibco-BRL). 50 μM 2-mercaptoethanol, and penicillin-streptomycin. Transfections were carried out as described previously (28).

Generation of FANCL, TAP-FANCL, and TAP-FANCL with sitetagged FANCF-TAP cell lines. The scheme of the generation of gene disruptions is outlined in Fig. 1A. The targeting construct for FANCL disruption was made by cloning the 5' arm, a KpnI/XhoI fragment amplified by PCR, with the primer pair 5'-CAGACGTTG CTAATGCGGT AAGACGCT and 5'-AGT ATCCACCATGT AATCCACATG CTCTTAGT AATTCTGAGT CAGGACCG TGGATCTGC T.

RESULTS

FANCL is the catalytic subunit of the FA core complex. In the chicken DT40 lymphoblastoid cell line, loss of the FA core complex protein FANCM results in the formation of unstable residual subcomplexes. Such subcomplexes have impaired E3 ligase activity and are defective in their accumulation in chromatin in response to DNA damage (26). To get further insight into the assembly and function of the FA core complex, we focused on FANCL, which probably provides the catalytic basis for the E3 ubiquitin ligase activity of the FA core complex. To study the function of FANCL in vivo, we first generated a DT40 strain where exons 2 to 6 (encoding the active cysteines Cys305 and Cys309 in the PHD/RING domain) were deleted (Fig. 1A). These cells are hypersensitive to the cross-linking agent cisplatin and are completely defective in their ability to monoubiquitinate FANC2 (Fig. 1B and C).

To study FA core complex assembly, we genetically engineered in situ a TAP (tandem affinity purification tag) cDNA into the FANCC locus in both wild-type and ΔFANCL strains (see Materials and Methods for details). To test the assembly of FANCC-TAP into the FA core complex, we monitored complex formation by size exclusion chromatography. In wild-type cells, FANCC-TAP eluted in fractions corresponding to a molecular mass of
~1.5 MDa. In contrast, the FANCC-TAP complex was much less abundant and shifted to fractions of a smaller size in ΔFANCL cells (Fig. 1D, top and middle elution profiles).

In many ways, the FANCL disruption is indistinguishable from disruptions of any of the other FA core complex genes in human or chicken cells. In all these instances the FA core complex fails to assemble. It is therefore impossible to establish whether FANCL enzymatic activity or its role in stabilizing the FA core complex results in the cellular FA defects. We created a mutant FANCL cell line that could support complex assembly but was deficient in the monoubiquitination of FANCD2. The FANCL protein domain structure consists of three N-terminal WD40 repeats and a C-terminal RING-like PHD domain (20). Although the isolated PHD/RING domain is capable of autoubiquitination in vitro, these domains can also function in chromatin interactions and phosphoinositide

FIG. 1. Functional separation of the FA core complex assembly and its E3 ubiquitin ligase activity. (A) Schematic representation of the genomic locus of FANCL with the exon configuration in correlation with the domain structure and the gene disruption construct with exons 2 to 6 removed. S, StuI. Southern blot analysis was performed on StuI/KpnI-digested genomic DNA from heterozygous and homozygous genotypes. WT, wild-type locus; Δ, gene knockout locus. The position of the probe used is indicated by the bar. (B) Sensitivity curves of the indicated cell lines. Cells were treated with various concentrations of cisplatin for 72 h and analyzed for survival in an MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] assay. Standard errors of the means are given from three independent experiments. (C) Immunoblot analysis of FANCD2 in subfractionated ΔFANCL cells either untreated (−) or treated with 150 ng/ml MMC (+). D2, FANCD2; D2-Ub, monoubiquitinated FANCD2; NEX, high-salt nuclear extract; CHEX, solubilized chromatin extract. (D) Nuclear extracts of the indicated cell lines (WT/C-TAP, ΔFANCL/C-TAP, and ΔFANCL/C-TAP + L357) were separated by size exclusion chromatography, and fractions were analyzed for FANCC-TAP and TAP-FANCL357 by immunoblot analysis. (E) Size exclusion chromatography of nuclear extracts of the indicated cell lines and immunoblot analyses for TAP-FANCC. (F) Subfractionation of either MMC-treated (+) or untreated (−) cells into high-salt NEX and CHEX and immunoblot analysis for FA2CD and TAP-FANCL. D2, FANCD2; D2-Ub, monoubiquitinated FANCD2. Histone H3 was used as a CHEX loading control. (G) Coimmunoprecipitation of wild-type and mutant TAP-FANCL and immunoblot analysis for FANCG. Nontagged wild-type cells were used as a negative immunoprecipitation control. FANCG antibody cross-reacts with the protein A domain of TAP-tag. WB, Western blot; S/N, supernatant of nonprecipitated proteins; P, fraction of precipitated proteins; Ig-H, immunoglobulin heavy chain.
binding. A recent study indicated that the WD40 repeats of FANCL may anchor this protein into the FA core complex (9). We mutated two highly conserved cysteine residues into alanines in the PHD/RING domain (C305A and C357A). DNAs encoding TAP-tagged wild-type FANCL as well as TAP-tagged mutants FANCL-C305A and FANCL-C357A were stably expressed in the ΔFANCL strain. TAP-FANCL point mutants failed to complement the cisplatin sensitivity and loss of FANCD2 monoubiquitination in the ΔFANCL strain (Fig. 1B and F). Next, we studied the migration pattern of TAP-FANCL in the ΔFANCL-complemented strains. Indeed, wild-type TAP-FANCL, TAP-FANCL-C305A, and TAP-FANCL-C357A coeluted at 1.5-MDa fractions, indicating that they form a stable FA core complex (Fig. 1E). In addition, we introduced TAP-FANCL-C357A into the ΔFANCL/FANCC-TAP strain. Size exclusion chromatography analysis clearly showed that FANCC-TAP coelutes with TAP-FANCL-C357A (Fig. 1D, bottom elution profile). Coimmunoprecipitation of FANCG with wild-type and point-mutated TAP-FANCL gave supporting evidence for the presence of an intact complex (Fig. 1G).

The FA core complex accumulates on chromatin following DNA damage, a response that requires the FANCM protein in chicken cells (26). We therefore tested whether the TAP-FANCL mutant FA core complexes were also able to respond to DNA damage in this manner. Following exposure to the DNA cross-linking agent mitomycin C (MMC), wild-type and mutant TAP-FANCL accumulated in the chromatin fraction (Fig. 1F). We also noted that unmodified FANCD2 accumulated on chromatin in a DNA damage-dependent manner. Furthermore, this accumulation is independent of functional FANCL. Taken together, our observations provide strong supportive evidence that the PHD/RING finger domain of FANCL is the catalytic subunit of the FA core complex.

Establishing a multitagged FA core complex in DT40 cells. The experiments described above indicate that it is possible to dissociate FA core complex assembly from its enzymatic activity. As mentioned earlier, studies from patient-derived cell lines carrying mutations in FA core complex genes have shown the presence of subcomplexes. These studies further predict that a regulated sequential assembly process may underpin temporal regulation of the FA pathway. We therefore asked if sequential complex assembly determines where and how the pathway is activated. A key factor that limits the study of the core complex is the lack of antibodies of sufficient sensitivity and specificity to FA proteins. To circumvent this, we created a DT40 strain that only expresses a multiple-tagged FA core complex. This strain contains in situ-tagged FANCC (FANCC-TAP), FANCF (FANCF-2xFLAG) (described recently in reference 26), and FANCB (FANCB-3xHA) genes (Fig. 2A). These tags were engineered directly into the genomic locus so that the tagged FA gene is under normal physiological regulation. Gene targetings were confirmed by Southern blot analyses (Fig. 2A). The triple-tagged FA core complex cell line is indistinguishable from wild-type cells in terms of cisplatin sensitivity (Fig. 2B) and its ability to monoubiquitinate FANCD2 (Fig. 2C). Next, we determined whether the tagged FA core complex proteins are assembled in a large complex. Size exclusion chromatography of nuclear extracts clearly showed that FANCC-TAP, FANCF-FLAG, and untagged FANCG coelute in fractions that correspond to a 1.5-MDa complex (Fig. 2D). To emphatically establish that these fractions contain the FA core complex, a coprecipitation with FANCC-TAP from 1.5-MDa fractions was performed. The analyses for the presence of FANCC-TAP, FANCM, FANCB-HA, FANCN, and FANCF-FLAG confirmed a stable complex in the 1.5-MDa size exclusion chromatography fractions (Fig. 2D).

The multitagged FA core complex is constitutively assembled. In order to study FA core complex formation during the cell cycle, the multitagged cell line was synchronized by nocodazole block in M phase and released into fresh medium. Synchronized cell populations were monitored and harvested at different time points as they transited through the G1, S, and G2/M phases of the cell cycle (Fig. 3A). Immunoprecipitations of FANCC-TAP from whole-cell lysates and immunoblot analyses of coprecipitated FA core complex components revealed that the FA core complex does not differ in its constituents in any of these phases, whereas FANCD2 monoubiquitination is at a maximum in late S/G2 (Fig. 3B and C).

Another means by which the pathway can be activated is by exposure to DNA-damaging agents. Cells were released from nocodazole block in the presence of HU, an agent known to induce stalled replication forks during S phase (Fig. 3D). FANCC-TAP was immunoprecipitated from either high-salt nuclear and soluble chromatin extracts of G1-phase cells (when FANCD2-Ub is not detectable) or of S-phase cells (with maximal detectable FANCD2-Ub levels) (Fig. 3E). Immunoblot analysis showed no difference in the composition or abundance of FA core complex constituents (Fig. 3F). We did, however, notice that the FANCM protein resolves as two distinct bands as cells transit from late S to G2 and M phases (Fig. 3B) and that this protein in nuclear extracts is up-shifted to a slightly lower mobility band following HU treatment and S-phase entry (Fig. 3F). These studies show that FANCC-TAP is associated with most of the FA core complex components.

To determine whether FANCC-TAP complexes are indeed intact FA core complexes and not subcomplexes, we carried out size exclusion chromatography experiments. Indeed, a prominent portion of FANCC-TAP elutes at 1.5 MDa in samples prepared from nuclear and chromatin extracts obtained from both G1- and S-phase-transiting cells (Fig. 4A). We did, however, notice that the FANCC-TAP elution peak of G1 chromatin extracts showed a broader distribution, which may reflect a less stable FA core complex. In addition to the fractionation of synchronized cells, we also induced DNA damage in asynchronous cells by exposing them to MMC. Once again, we detected no obvious difference in the size of the FANCC-TAP complex in extracts prepared from both compartments following this treatment (Fig. 4B). However, the amount of FANCC-TAP on chromatin in the absence of MMC is very low and can therefore hardly be detected in 1.5-MDa fractions. In conclusion, a 1.5-MDa FA core complex is assembled regardless of DNA damage or cell cycle transitions.

The FA core complex is stable and accumulates on chromatin in the absence of UBE2T. Most gene products that are essential for the E3 ligase activity of the FA core complex are also integral components of this complex. One exception is the recently identified FANCl gene, which is not essential for the stability of the core complex but is required for FANCD2 monoubiquitination. However, it is unclear whether UBE2T,
the E2-conjugating enzyme for FANCD2 monoubiquitination, is also required for the stability of the FA core complex and its chromatin accumulation after DNA damage. To address this, we disrupted the UBE2T gene in DT40 cells. The gene disruption construct is outlined in Fig. 5A and removes exons 4 to 6, including the active cysteine Cys86. Two independently derived ΔUBE2T cell lines show hypersensitivity to cisplatin (Fig. 5B) and are defective in the monoubiquitination of FANCD2 (Fig. 5D). To determine whether the FA core complex is compromised in the absence of UBE2T, we fractionated cell extracts from wild-type and ΔUBE2T cells and analyzed these fractions for the elution profile for FANCG. FANCG can be detected in the 1.5-MDa fractions in the ΔUBE2T cell line, indicating that the FA core complex is intact and stable (Fig. 5C), like in wild-type cells. We then tested to see if UBE2T is required for the FA core complex or FANCD2 to accumulate on chromatin. Following exposure to MMC, wild-type cells demonstrate robust FANCD2 monoubiquitination and both FANCD2 and FANCG accumulate on chromatin (Fig. 5D). FANCD2 is not monoubiquitinated in ΔUBE2T strains; however, FANCD2 and the FA core complex subunit FANCG still accumulate on chromatin following damage (Fig. 5D). In conclusion, UBE2T is essential for FANCD2 monoubiquitination but is not required for either FA core complex stability or accumulation on chromatin.

UBE2T accumulates constitutively on chromatin. The FA core complex and UBE2T must form a transient holoenzyme complex on chromatin for FANCD2 monoubiquitination to occur. We therefore determined how UBE2T gains access to this compartment. When we tested nuclear and
soluble chromatin extracts from cells exposed to MMC, we noted that UBE2T is constitutively present on chromatin (Fig. 5F). Moreover, we compared extracts from G₁ to S phase for the presence of UBE2T and FANCD2. UBE2T always fractionated with chromatin, whereas monoubiquitinated FANCD2 accumulated on chromatin during the S phase only (Fig. 5E). Cumulatively, these studies indicate that UBE2T is predominantly localized on chromatin in...
DT40 cells independently of DNA damage or cell cycle transitions.

**DISCUSSION**

The main conclusion of this study is that a constitutively assembled FA core complex with an associated E3 ubiquitin ligase activity responds to DNA damage by migrating to chromatin. Here, the FA core complex likely interacts with UBE2T and FANCD2. These two proteins access chromatin independently of the FA core complex. An important implication of this work is that in chicken B cells the FA core complex E3 ubiquitin ligase activity is not regulated by an assembly/disassembly cycle of the FA core subunits. This is an unexpected conclusion, as studies on FA patient-derived cell lines led to the hypothesis that the sequential assembly of this complex underpins the regulation of FANCD2 monoubiquitination (17, 19). In addition, early studies indicated that some of the FA proteins were regulated in a cell cycle-dependent manner (10). However, we found that the assembled FA core complex is very stable. It would seem to be wasteful, from the point of view of a cell's energy, to disassemble such a complex when it is not needed, only to reassemble it later. Furthermore, many of the FA core complex proteins are of very low abundance in cells, suggesting that assembly of the complete FA core complex may be limited by the concentration of its constituents. An important caveat is that our studies in chicken DT40 cells may not apply to mammalian cells. Nevertheless, having concluded that the FA core complex is not regulated by an assembly process checkpoint, there are certain points of discussion raised by the presence of such a constitutive FA core complex. How does it respond to DNA damage and how does it get recruited to chromatin? What enables such a large complex to be retained or gain access to the nucleus after mitosis is completed? Finally, how stable is this complex, and does it turn over rapidly once activated or is it recycled?

The first point we discuss here is the model we have put forward explaining how the activity of the FA core complex might be restricted (Fig. 5G). Central to the model is that the E3 ubiquitin ligase, the FA core complex, and the substrates FANCD2/FANCI independently accumulate on chromatin during DNA replication or damage. The E2-conjugating enzyme UBE2T is constitutively present in this compartment. Hence, it can then form a transiently active E2/E3 holoenzyme for the monoubiquitination of FANCD2/FANCI. How the FA core complex and its substrate FANCD2/FANCI translocate to chromatin needs to be established. We have recently shown that the FANCM component of the FA core complex is required not only for the FA core complex to assemble but also for it to accumulate on chromatin (26). In addition, this protein has two domains that provide it with an ability to recognize DNA structures that occur at stalled DNA replication forks (13, 14). It has recently been shown that this interaction of FANCM with DNA structures is mediated by the FA core complex component FAAP24 that binds to the C-terminal part.
of FANCM (4). How the interaction is regulated is still an open question, but FANCM is also hyperphosphorylated upon DNA damage, and it is plausible that this modification alters its affinity or activity towards DNA (21). The manner in which FANCD2 gains access to the chromatin compartment deserves attention. In this study, we did find a requirement for a stable FA core complex for nonubiquitinated FANCD2 to accumulate on chromatin. However, it was clear that monoubiquitin-
ated FANCD2 did strongly associate with chromatin. Biochemical studies suggest that FANCD2 is able to bind directly to DNA (30). In addition, this protein is phosphorylated by both ATR and ATM DNA checkpoint kinases, which are induced by different kinds of DNA lesions (1, 37). Since disruption of some of the phosphorylation sites weakens the ability of FANCD2 to be ubiquitinated and to accumulate in DNA damage-induced foci, it is therefore possible that these modifications are critical signals that allow the unmodified protein to transiently accumulate on chromatin. In addition, a recent study provides evidence that γ-H2AX at sites of DNA damage is essential for the accumulation of FANCD2 into foci (2). Future studies using single point mutants of key FA core complex proteins and FANCD2 should enable a dissection of how both components accumulate on chromatin.

At mitosis, the nuclear envelope breaks down and essentially the strict demarcation between nucleus and cytoplasm ceases to exist. The FA core complex is a large complex and as such is present in low abundance in cells. A key problem raised by the current work is how a constitutively assembled complex gains access to a newly formed nucleus. It is very unlikely that nuclear import of FA core complexes that are dispersed in the cytoplasm occurs after mitosis. Because of the molecular size, ~1.5 MDa, of this complex, it would seem beyond the capacity of the nuclear pore size to allow free diffusion of the FA core complex. One could imagine that the FA core complex is tethered to a structural component (such as nuclear lamin, lipid membrane, or indeed, condensed chromosomes) at mitosis; thus, when the nucleus reforms it is carried into the structure directly. This model is supported by recent structural insights into FANCE. This FA protein has a putative membrane-adsorbing amphipathic α-helix which is shared among some membrane-associated proteins and which is thought to recognize curved lipid membranes (7). Hence, FANCE might be the anchor for the FA core complex to remain associated with nuclear membrane subunits during mitosis. Another possibility is that natural loss of the FA core complex occurs, and only by some virtue of even distribution does it get captured into a new nucleus. If this were the case, a high turnover rate of core complex formation might be expected.

The ability to monitor a physiologically tagged FA core complex should enable us to establish its natural turnover. While we could not detect any major differences in the steady-state levels of the FA core complex during DNA replication or DNA damage, this does not give us much idea regarding the rate of turnover. It is important to note that the work presented here does not deny the existence of subcomplexes. Such subcomplexes have been detected for certain pairs of FA proteins and could be intermediates of a subsequent assembly (17, 19). Another possibility is that these subcomplexes might confer unique and distinct biochemical functions. Since ablation of any one of the FA core complex proteins severely compromises the complex stability, as such, as well that of individual FA proteins, it remains possible that activated FA core complexes may be less stable. Moreover, as more components of the FA core complex are identified, it will be possible to test their contribution to stability by gene ablation in the multiple-tagged FA core complex cell line. Future studies to determine the precise half-lives and turnover rates of the FA core complex and complex components may evolve to lead important insights into the function of the FA core complex.

ACKNOWLEDGMENTS

A.A. was supported by the Leukemia Research Fund and F.L. by the Children with Leukemia Fund.

We are grateful to Paul Pace and other members of K. J. Patel’s laboratory for critical comments and technical advice.

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