Functional Interaction of bZIP Proteins and the Large Subunit of Replication Factor C in Liver and Adipose Cells*

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The transcription factor CCAAT/enhancer-binding protein-α (C/EBPα) has a vital role in cell growth and differentiation. To delineate further a mechanism for C/EBPα-mediated differentiation, we screened C/EBPα-interacting proteins through far-Western screening. One of the strongest interactions was with RFC140, the large subunit of the replication factor C complex. C/EBPα specifically interacted with RFC140 from rat liver nuclear extract as determined by a combination of affinity chromatography and co-immunoprecipitation. Subsequent far-Western blotting showed that the bZIP domain of C/EBPα interacted with the DNA-binding region of RFC140. Overexpression of RFC140 in mammalian cells increased the transactivation activity of C/EBPα on both minimal and native promoters. Consistent with the enhanced transactivation, a complex of C/EBPα and RFC140 proteins with the cognate DNA element was detected in vitro. The specific interaction between C/EBPα and RFC140 was detected in the terminal differentiation of 3T3-L1 preadipocytes to adipocytes. The synergistic transcription effect of these two proteins increased the promoter activity and protein expression of peroxisome proliferator-activated receptor-γ, which is a main regulator of adipocyte differentiation. Our results demonstrate that the specific transcription factor C/EBPα and the general DNA replication factor RFC140 interact functionally and physically. This observation highlights a unique mechanism by which the levels of the general replication factor can strongly modulate the functional activity of the specific transcription factor as a coactivator.

Transcription factors, regulatory proteins whose activities are tightly controlled, have been referred to as a paradigm for modular proteins. Minimally, these factors encode a DNA-binding domain and a transcription-activating domain. Additional functional modules were defined by studies analyzing the regulation of transcription factor activity. For example, factors in the nuclear receptor superfamily encode a ligand-binding domain that renders their function hormone-dependent. The striking feature that is shared among these protein modules is their ability to function when transferred to another protein (1). Furthermore, structure/function experiments showed that some protein modules, e.g. transactivation domains, function by providing a surface for interacting with other proteins.

The transcription factor C/EBPα contains a bZIP module and is constitutively expressed in fat cells and adult organs such as liver and lung. A C/EBPα homodimer binds the DNA major groove through α-helices that are held in register by a classical coiled-coil motif, the leucine zipper (2, 3). C/EBPα activates transcription of genes that typify the differentiated cell phenotype, most notably in liver and fat cells (4–6). Interestingly, a relationship between expression of C/EBPα and cell growth control has been established (7–9). Fibrolasts transfected with a C/EBPα expression plasmid were observed to withdraw from the cell cycle (10). Subsequently, a role for C/EBPα in fat cell differentiation was revealed. A hormonal regimen that initiates differentiation of 3T3-L1 cells results in C/EBPα expression coincident with conversion of preadipocytes into fat-producing cells in culture (11–14). In fact, constitutive overproduction of C/EBPα induces growth arrest of several fibroblastic cell lines and in some cases is sufficient to promote adipose conversion (15), suggesting that C/EBPα is a component of a differentiation switch. An essential role for C/EBPα during fat cell differentiation was documented by expressing an antisense C/EBPα construct in 3T3-L1 cells that blocked adipose conversion. Furthermore, homozygous disruption of the C/EBPα gene is characterized by accumulation of undifferentiated fat cells prior to prenatal death (16). Recent reports demonstrated a requirement for the Rb protein in the differentiation of fat cells and showed that Rb can interact with C/EBPα proteins (17–19). Another report showed that the cyclin-dependent kinase inhibitor WAF1 plays a role in growth arrest following C/EBPα expression in a cultured fibrosarcoma cell line (10). To learn more about the role that C/EBPα plays in cell differentiation, we took advantage of the modular nature of the protein.

Activator proteins such as C/EBPα bind specific DNA sequences located either upstream or downstream of the core promoter. In response to physiological cues, activators stimulate transcription initiation by interacting with general transcription factors, with TATA-associated factors, or with coactivators. We utilized a radiolabeled GST-C/EBPα fusion protein to screen C/EBPα-interacting proteins using an expression cDNA library prepared from rat liver mRNA. One of the cDNAs

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‡ The abbreviations used are: C/EBP, CCAAT/enhancer-binding protein; bZIP, basic region leucine zipper; Rb, retinoblastoma protein; GST, glutathione S-transferase; RFC, replication factor C; MBP, maltose-binding protein; CRE, CAMP-responsive element; CREB, CAMP-responsive element-binding protein; NF-κB, nuclear factor-κB; PPARγ, peroxisome proliferator-activated receptor-γ; PCNA, proliferating cell nuclear antigen.
we isolated encoded the large subunit of RFC. We mapped the interacting domain of each protein and show that transient transfection of RFC140 has an increased effect on the transactivation activities of C/EBPα. Furthermore, the functional interaction of C/EBPα and RFC140 may be necessary for adipocyte differentiation.

**EXPERIMENTAL PROCEDURES**

**Cloning of cDNAs Encoding C/EBPα-Interacting Proteins—**A Agt11 cDNA library prepared from rat liver mRNA (CLONTECH) was plated at a density of 30,000 plaques/150-mm plate as described (21). Briefly, phage were plated, incubated at 42 °C for 1 h, and washed twice with buffer B, and solubilized with 20 ml of 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, and 0.1% SDS-polyacrylamide gel and transferred to nitrocellulose (0.45 μm; BA85, Schleicher & Schuell). The filter was blocked for 1 h in Hyb75 containing 5% nonfat milk. Radiolabeled GST-C/EBPα was added to 25 ml/filter Hyb75 containing 5% nonfat milk and 100,000 cpm/ml labeled probe overnight at 4 °C. Filters were washed three times for 5 min each at 4 °C in 50 ml/filter Hyb75 containing 0.25% nonfat milk. Filters were air-dried briefly and exposed to film.

**Construction, Expression, and Purification of GST Fusion Proteins**—The vector pGEX-2T (Amerham Pharmacia Biotech) was modified by the insertion of a protein kinase A phosphorylation site as described (22). In-frame fusions were prepared from C/EBPαΔ1–2 (3) by standard cloning techniques. Constructs lacking the leucine zipper and the bZIP domain were prepared by placing in-frame stop codons at amino acids 310 and 272, respectively. MinI digestion and fill in created the fusion to amino acid 192. Constructs expressing amino acids 281–358 and 281–342 have been described (23). Fusion proteins are designated according to the C/EBPα amino acids that they contain and are shown schematically in Fig. 2. Detailed construction of these plasmids has been described (23, 38). RFC140 fusion proteins were prepared from the full-length mouse RFC cDNA (kindly provided by Dr. Yoshihiko Yamada, National Institutes of Health). The pair of oligonucleotide primers generated a 5’-BamHI site and a 3’-EcoRI site for cloning. Expression plasmids were transformed into host strain BL21, grown to an A600 of 0.8, and induced with 1 mM isopropyl-β-D-thiogalactopyranoside for 2 h. Bacteria were harvested by centrifugation, lysed by sonication, and purified by anion exchange using glutathione-agarose beads (Amerham Pharmacia Biotech) according to the manufacturer’s instructions. Equivalent amounts of purified fusion proteins were determined by SDS-polyacrylamide gel electrophoresis and Coomassie Blue staining.

**Affinity Chromatography**—Equivalent amounts of MBP or MBP-RFC140 were coupled to Sepharose 4B. 100-μl columns were equilibrated in Hyb50 (50 mM Tris (pH 8.0), 150 mM KCl, 0.1 mM EDTA, 2.5 mM MgCl2, 5 mM 2-mercaptoethanol, and 0.1% Nonidet P-40). 70 μg of rat liver nuclear extract was passed over each column, followed by a 15-volume wash with Hyb50. Columns were eluted stepwise with 1.8 volumes of buffer containing 0.25, 0.5, and 1.0 M KCl. Equivalent percentages of the unbound and eluted fractions were loaded onto 15% SDS-polyacrylamide gels, separated, and electroblotted for Western blot analysis. The presence of C/EBPα was detected with anti-C/EBPα antiserum.

**Preparation of Antiserum**—The MBP-RFC140 fusion protein was expressed in 1 liter of broth and purified on maltose beads (New England Biolabs Inc.). Purified antigen was excised from a 15% SDS-polyacrylamide gel, and antiserum was prepared according to standard procedures as recommended by Spring Valley Labs.

**Western and Modified Western Blot Analyses**—Equivalent amounts of purified truncated C/EBPα proteins were fractionated on a 12% SDS-polyacrylamide gel and transferred to nitrocellulose (0.45 μm; BA85, Schleicher & Schuell). The filter was blocked for 1 h in binding buffer A (20 mM Tris (pH 8.0), 120 mM NaCl, 0.1 mM EDTA, 2.5 mM MgCl2, 0.2% Nonidet P-40, and 1 mM dithiothreitol) supplemented with 0.5% nonfat milk. Purified GST-RFC140 (1 μg) was added to binding buffer A and incubated with rocking for 30 min at room temperature. The solution was decanted, and filters were washed three times with fresh binding buffer A. Bound RFC140 was detected with anti-RFC Ig (12 ng/ml) followed by horseradish peroxidase-conjugated donkey anti-rabbit IgG (1:5000), and visualized by chemiluminescence using ECL reagent (Amerham Pharmacia Biotech). Filters were exposed to Eastman Kodak X-OMAT AR film. Similarly, equivalent amounts of purified GST-RFC140 fusion proteins were separated and electroblotted onto nitrocellulose membrane. The filters were incubated with purified GST-C/EBPα and washed, and bound C/EBPα was detected with specific anti-EBPα antisera essentially as described above.

**Immunoprecipitation and Co-immunoprecipitation Analyses—**C/EBPα was immunoprecipitated from 200 μg of rat liver nuclear extract by incubation with 15 μl of anti-C/EBPα IgG in a total volume of 500 μl of binding buffer B (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, and 10% glycerol). After rocking for 1 h at 4 °C, 20 μl of protein A-Sepharose beads (1:1 slurry; Amersham Pharmacia Biotech) was added. The sample was rocked for 1 h at 4 °C, washed five times with binding buffer B, and solubilized with 20 μl of sample buffer. The eluted proteins were separated on a 10% SDS-polyacrylamide gel and electroblotted, and coprecipitating RFC140 was detected by Western blotting with anti-RFC140 IgG. Secondary detection was carried out with horseradish peroxidase-conjugated donkey anti-rabbit IgG, followed by chemiluminescent development with ECL reagent.

**Transient Transfection Analysis**—Subconfluent Fao cells (kindly provided by Mary Weiss, Institut Pasteur) were transfected by the standard calcium phosphate technique. 48 h after transfection, cells were washed twice with phosphate-buffered saline, harvested, and lysed in 250 μl of reporter lysis buffer. Lysates were clarified by centrifugation at 15,000 rpm for 5 min in an Eppendorf microcentrifuge, and relative luciferase activity was determined using the luciferase assay system (Promega). All transfections included pCMV-β-galactosidase, and relative β-galactosidase activity was determined using the β-galactosidase enzyme assay system (Promega) to normalize results for transfection efficiency.

**Electrophoretic Mobility Shift Assay**—GST fusion proteins were purified according to the protocol described above. Equal amounts of GST-C/EBPα (3098) protein (50 μg) were incubated with 32P end-labeled CRE oligonucleotides in the presence or absence of GST-RFC140 (1–151) or GST-full-length RFC140. Binding reactions were performed in a 20-μl volume containing increasing amounts of GST-RFC140 proteins, 4 μl of 5 × binding buffer C (20 mM HEPES (pH 7.5), 50 mM KCl, 1 mM dithiothreitol, 2.5 mM MgCl2, and 20% Ficoll), 2 μg of CRE probe as nonspecific competitor DNA, 2 μg of bovine serum albumin, and 10,000–15,000 cpm of radiolabeled oligonucleotide. After 30 min of incubation at room temperature, samples were loaded onto an 8% nondenaturing polyacrylamide gel in 0.5× Tris borate/EDTA buffer (pH 8.3). After electrophoresis, gels were dried and exposed to x-ray film.

**Induction of Adipocyte Differentiation**—3T3-L1 fibroblasts were differentiated into adipocytes by reaching confluence by the addition of differentiation medium (high-glucose Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, 1 mg/ml-glutamine, 0.5 mM isobutylmethylxanthine, 1 μM dexamethasone, and 1 μg/ml insulin). After 2 days, the 3T3-L1 cells were transferred to adipocyte growth medium (high-glucose Dulbecco’s modified Eagle’s medium plus 10% fetal calf serum, 1 mg/ml-glutamine, and 1 μg/ml insulin) and refed every 2 days. Differentiation of fibroblasts to mature adipocytes was confirmed by oil red O staining of lipid vesicles.

**RESULTS**

**RFC140 Associates with C/EBPα in Vitro**—We identified proteins that physically associate with C/EBPα by interacting a radiolabeled GST-C/EBPα fusion protein with proteins expressed from a liver Agt11 cDNA library (21). During this screen, which was confirmed through 5′-selective far-Western blot hybridization procedures, we purified a cDNA encoding RFC140. The cDNA insert was subcloned for bacterial expression as a fusion to MBP, and specific antiserum was raised. To more rigorously test the specific interaction between RFC140 and C/EBPα, we chromatographed fresh rat liver nuclear extract on an MBP-RFC140 affinity column. As a specificity control, an MBP affinity column was run in parallel. After extensive washing, bound proteins were eluted, gel-fractionated, and analyzed by Western blotting with anti-C/EBPα antiserum. Endogenous C/EBPα migrated as a 42-kDa protein and a 30-kDa internal translation initiation product (Fig. 1, first lane). The MBP-RFC140 affinity column bound most of the C/EBPα in the nuclear extract (second lane), and the interac-
tion was stable up to 0.25 M KCl, requiring 0.5 M KCl for elution (fifth lane). In contrast, C/EBPα was found exclusively in the unbound fraction of the MBP affinity column (ninth lane). These results suggest that the interaction of RFC140 with C/EBPα occurs with specificity.

Co-immunoprecipitation of Endogenous RFC140 with C/EBPα—The association between C/EBPα and RFC140 was further characterized by co-immunoprecipitation analysis. C/EBPα was immunoprecipitated from freshly prepared rat liver nuclear extracts, gel-fractionated, and analyzed for RFC140 coprecipitation by Western blotting with anti-RFC140 IgG. As shown in Fig. 1B (fourth lane), endogenous RFC140 was detected as a coprecipitant in C/EBPα immunoprecipitates. A parallel immunoprecipitate formed with preimmune serum failed to show RFC140 immunoreactivity (second lane). Compared with RFC140 immunoprecipitates (third lane), ~10% of endogenous RFC140 protein could be calculated to interact with C/EBPα. These results are consistent with the interpretation that C/EBPα is capable of associating with RFC140 in vitro and in vivo.

The DNA-binding Domain of RFC140 Associates with the bZIP Domain of C/EBPα—To determine the protein domains mediating association between these nuclear factors, sequential deletion constructs were expressed as GST fusion proteins, purified, and analyzed for protein interaction using a modified Western blot technique. Equivalent amounts of deletion proteins of C/EBPα or RFC140 were separated on SDS-polyacrylamide gels and transferred to nitrocellulose membrane. The blot was incubated with purified GST-RFC140 or GST-C/EBPα, washed extensively, and subsequently developed with RFC140- or C/EBPα-specific antibodies, respectively. As shown in Fig. 2A (second through fourth and seventh lanes), soluble RFC140 bound to membrane-bound C/EBPα deletion proteins if the DNA-binding domain and leucine zipper were present. In addition, weaker association was observed when the leucine zipper was removed (sixth lane), suggesting that interaction is not solely mediated by a coiled-coil interaction. The reciprocal strategy was used to delineate the region of RFC140 required for interaction with C/EBPα. As shown in Fig. 2B, RFC140 (151–360), the original cDNA insert, bound C/EBPα. Surprisingly, the truncated protein encompassing amino acids 361–545 bound C/EBPα more efficiently. This construct encompasses the DNA-binding/DNA ligase-like domain of RFC140 (amino acids 369–480), and any truncated protein containing this domain bound C/EBPα efficiently (third through fifth lanes). In contrast, the amino-terminal (second lane) and carboxyl-terminal (sixth lane) halves of the protein were dispensable for interaction with C/EBPα. These results indicate that the bZIP domain of C/EBPα is involved in the association with RFC140 through the DNA-binding/DNA ligase-like domain.

RFC140 Interacts with Other bZIP Proteins—As shown in Fig. 2, RFC140 interacted with C/EBPα through the bZIP domain. This led us to examine whether RFC140 interacts with other bZIP proteins. The N-terminal region (amino acids 1–545) including the C/EBPα-binding domain and the C-terminal region (amino acids 546–1148) of RFC140 were purified from Escherichia coli as GST fusion proteins. The proteins were immobilized on glutathione-Sepharose beads and incubated with in vitro translated 35S-labeled C/EBPα, C/EBPβ, CREB, activation transcription factor-2, c-Jun, NF-κB (p65), and retinoic acid receptor proteins. C/EBPα and C/EBPβ strongly associated with the N-terminal region of RFC140, but not with the C-terminal region (Fig. 3). In addition to these specific physical associations of RFC140 with members of the C/EBP family protein family, the N-terminal truncated RFC140 protein interacted with CREB, activation transcription factor-2, and c-Jun. Other structural transcription factors, p65 of NF-κB and the retinoic acid receptor, did not interact with RFC140. This suggests that RFC140 specifically interacts with all bZIP proteins.

The RFC140 Subunit Affects Transactivation by C/EBPα in a Transient Assay—If C/EBPα and RFC140 associate, overproduction of RFC140 might affect C/EBPα-dependent transactivation activity. To test this, the hepatoma cell line Faos was transfected with expression plasmids encoding C/EBPα and RFC140, along with a luciferase reporter driven by the minimal thymidine kinase promoter containing two proximally inserted C/EBPα-binding sites. As shown in Fig. 4A, transfection of RFC140 alone did not significantly affect basal reporter activity, whereas transfection of C/EBPα alone stimulated the minimal promoter construct ~3-fold. When cells were cotransfected with a constant amount of C/EBPα, the activity of the
minimal promoter increased in an RFC140 dose-dependent fashion. To demonstrate that the enhanced transactivation by RFC140 was dependent upon C/EBPα binding, the two C/EBPα-binding sites in the reporter plasmid were mutated, and the experiments were repeated. Since C/EBPα and RFC140 did not show increased transactivation activity with the mutated reporter plasmid (data not shown), this shows that C/EBPα-mediated co-transactivation by RFC140 is dependent on C/EBPα binding to its sequence. Overproduction of RFC140 was confirmed by Western analysis (see Fig. 4C for representative results).

We also determined whether RFC140 had similar effects on transactivation of the native phosphoenolpyruvate carboxykinase promoter (−275 to +55) in addition to the minimal promoter. Phosphoenolpyruvate carboxykinase expression was high in liver and adipose cells. C/EBPα was reported to bind the CRE sites in this promoter, stimulating its transcription (39). As shown in Fig. 4B, transfected C/EBPα stimulated transcription from the phosphoenolpyruvate carboxykinase (−275 to +55) promoter construct. Upon cotransfection of RFC140, activation increased from ~6- to ~14-fold. Taken together, these results are consistent with the interpretation that the association of C/EBPα with RFC140 potentiates the transactivation activity of C/EBPα.

**Figure 2.** The DNA-binding domain of RFC140 associates with the bZIP domain of C/EBPα. A, the bZIP domain of C/EBPα is required for interaction with RFC140. Equivalent amounts of truncated GST-C/EBPα fusion proteins (affinity-purified) were separated by SDS-polyacrylamide gel electrophoresis, electrobotted, and transferred to Immobilon membrane. After blocking in 5% milk, fresh blocking buffer A was supplemented with purified GST-RFC140 (1 μg). After 1 h, the filter was washed, and bound RFC140 was detected with anti-RFC140 IgG and horseradish peroxidase-conjugated donkey anti-rabbit IgG. The fusion proteins are shown schematically below the blot. TAD, DBD, and DD indicate the transactivation domain (white bars), DNA-binding domain (stippled bars), and dimerization domain (black bars), respectively. The C-terminal domain of C/EBPα (hatched bars) does not have any known functional domain. B, the DNA-binding/DNA ligase-like domain of RFC140 is required for interaction with C/EBPα. Truncated GST-RFC140 proteins were fractionated, blotted onto a membrane, and incubated with soluble GST-C/EBPα. After washing, bound C/EBPα was detected with anti-C/EBPα IgG and horseradish peroxidase-conjugated donkey anti-rabbit IgG. Fusion proteins are shown schematically below the blot.

**Figure 3.** RFC140 interacts differently with other bZIP proteins. GST fusions to the N- and C-terminal regions of RFC140 were purified from E. coli. *In vitro* translated [35S]methionine-labeled proteins (C/EBPα, C/EBPβ, CREB, activation transcription factor-2 (ATF2), c-Jun, p65 (NF-κB), and the retinoic acid receptor (RAR)) were incubated with glutathione-resin-immobilized GST, GST-N-terminal RFC140 (GST/RFC140-N), and GST-C-terminal RFC140 (GST/RFC140-C). The bound proteins were eluted with reduced glutathione and resolved by SDS-polyacrylamide gel electrophoresis.
with the intensity of the supershifted band gradually increasing dependent on the added amounts of the full-length RFC140 protein. The RFC140 protein alone did not bind the CRE probe in the absence of C/EBPα (second through fourth lanes). These results suggest that the interaction of the RFC140 subunit with C/EBPα can induce a strong complex with the C/EBPα-binding DNA element, resulting in the increased C/EBPα-dependent transactivation by RFC140.

C/EBPα Specifically Interacts with RFC140 in Differentiating Adipocytes—The RFC140 protein is increased after induction of adipocyte lineage differentiation of 3T3-L1 cells by inducers (35). C/EBPα transient expression is known to appear upon the terminal differentiation of 3T3-L1 cells to adipocytes (11). These results suggest a possibility that C/EBPα has functional interaction with RFC140 in the adipocyte differentiation procedure. To examine whether the presence of a protein interaction between C/EBPα and RFC140 would be found in the differentiation process of adipocytes, we tried to identify the specific interaction of two proteins by co-immunoprecipitation. RFC140 was detected in the co-immunoprecipitated protein fractions by anti-C/EBPα antibody using differentiation-induced cell extract at the 5th and 7th days after inducer treatment (Fig. 5). This interaction was not found in undifferentiated cell extract and in the first 3-day extracts after differentiation induction. Fig. 4 results also show that the coexpression of C/EBPα and RFC140 increased the promoter activity of phosphoenolpyruvate carboxykinase, which is a representative marker protein for adipocyte maturation. These results suggest that the interaction of C/EBPα and RFC140 functions in the terminal differentiation of adipocytes in vivo.

The Synergistic Action of C/EBPα and RFC140 Promotes the Promoter Activity and Protein Expression of PPARγ—Lyle et al. (36) showed that the treatment of antisense oligonucleotides to RFC140 inhibits adipocyte maturation. These results suggest that the interaction of C/EBPα and PPARγ expression. For elucidating this, we investigated the promoter activity and protein expression of PPARγ after transient transfection of expression plasmids encoding C/EBPα and RFC140.
Functional Interaction of bZIP Protein and RFC140

**DISCUSSION**

We isolated the cDNA for RFC140 as an expressed product that interacts with C/EBPα. RFC is a five-subunit complex composed of 140-, 40-, 38-, 37-, and 36-kDa subunits (24). Although RFC140 is primarily responsible for DNA binding, each of the subunits shares a conserved domain that is referred to as the PCNA interaction region (25). In yeast, each individual subunit is an essential gene product that functions in the replicative holoenzyme complex (26, 27). It is intriguing to consider that DNA-binding activity may be affected. This was suggested by the observation that antisense p21 expression resulted in reentry of C/EBPα-expressing cells into the division cycle (28). Although the mechanism was shown to be primarily post-translational, it was concluded that p21 was responsible for C/EBPα-dependent growth arrest based upon the observation that antisense p21 expression resulted in reentry of C/EBPα-expressing cells into the division cycle (28). Although this may in part explain the observed growth arrest, it cannot be the complete story, as p21 is not an essential gene product.
During development, acquisition of the differentiated phenotype involves expression of function-specific genes along with entry of the cells into a quiescent phase of the cell cycle. C/EBPa, as well as MyoD, NF-xB, Zta, and JunD, was observed to inhibit cell division and to induce cell differentiation (29, 30). This is consistent with a differentiation model whereby cells first stop dividing and subsequently express transcripts that reflect the differentiated cell state. Interestingly, the antiproliferative effects exerted by certain transcription factors can be separated from transcription activation activity. For example, expression of MyoD in fibroblasts inhibits cell division without inducing expression of muscle-specific gene products (31).

The interaction of C/EBPa with RFC140 presents another mechanism by which C/EBPa could contribute to cell proliferation and differentiation. The association of C/EBPa with RFC140 could inhibit cell cycle progression by interfering with the loading of a PCNA clamp onto DNA. This is a plausible hypothesis, as it was previously shown that association of PCNA with p21WAF1 resulted in inhibition of the cell cycle (40). This occurred because the PCNA sliding clamp is essential for proccessive movement of the replicative DNA polymerase-δ complex. By binding to PCNA, p21 impedes proccessive DNA synthesis, but not short repair DNA synthesis. By analogy, it is possible that association of RFC140 with C/EBPa interferes with the DNA-binding activity of the RFC complex, with the folding of the pentameric complex, or with the ability of the RFC complex to load the PCNA clamp onto DNA, thereby leading to cell cycle arrest and further terminal cell differentiation. Currently in our laboratory, we are planning to examine whether the interaction of C/EBPa with RFC140 disrupts the DNA replication system and induces cell growth arrest.

Although the molecular mechanism(s) by which transcription factors affect growth control is not firmly established, there are insights. For example, overproduction of the Epstein-Barr virus product Zta leads to post-transcriptional induction of the p53 tumor suppressor protein and the cyclin-dependent kinase inhibitors p21WAF1 and p27KIP1 (41). Similarly, the half-life of p21WAF1 was observed to increase in cells growth-arrested after C/EBPa expression (10). The reciprocal outcome may be obtained as well, as the transactivation activity of NF-xB was reported to increase after transfection of a p21WAF1 expression plasmid (42). Thus, blocking the cell cycle may have a positive effect on the transactivation function of certain transcription factors as well.

Clement and co-workers (32) showed that RFC140 is more abundant in nuclear extracts from hepatoma cells treated with butyrate, which blocks the cells in the G1 phase of the cell cycle, than in those from routinely cultured cells and that nuclear distribution of the large subunit of RFC changes during the cell cycle. The RFC140 subunit has homology to CDC44, which is a functional and physical interact. This observation, along with those made previously, highlights a unique mechanism by which the levels of the general replication factor can strongly modulate the functional activity of the specific transcription factor as a coactivator in non-proliferating cells, especially in a differentiation procedure.

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