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Adipocyte-specific transcription factor ARF6 is a heterodimeric complex of two nuclear hormone receptors, PPARγ and RXRα

Peter Tontonoz, Reed A.Graves+, Adriane I.Budavari, Hediye Erdjument-Bromage[1], Mary Lui[1], Erding Hu, Paul Tempst[1] and Bruce M.Spiegelman*
Dana-Farber Cancer Institute and the Department of Cell Biology, Harvard Medical School, Boston, MA 02115 and [1]Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA

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ABSTRACT

Previously, we identified a novel transcription factor, ARF6, as a key regulator of the tissue-specific adipocyte P2 (aP2) enhancer. In order to identify the proteins which comprise the adipocyte ARF6 complex, we have purified this DNA binding activity from a cultured adipocyte cell line. We have developed a system for growth and differentiation of HIB-1B brown adipocytes in suspension culture that facilitates the production of large quantities of adipocyte nuclear extract. ARF6 was purified from HIB-1B nuclear extract by a combination of conventional and sequence-specific DNA affinity chromotography. Chemical sequencing and mass spectral analysis of tryptic peptides derived from the purified polypeptides identifies the ARF6 complex as a heterodimer of the retinoid X receptor α (RXRα) and the murine peroxisome proliferator activated receptor γ (PPARγ). Of the known PPARγ isoforms, PPARγ is the predominant form expressed in adipose tissue. These results suggest that PPARγ2 serves a unique function among PPAR family members as an important regulator of adipocyte-specific gene expression.

INTRODUCTION

The murine adipocyte P2 (aP2) gene encodes an intracellular lipid binding protein and is expressed specifically in adipose cells. We have been studying the transcriptional regulation of this gene in an effort to understand the molecular basis for adipocyte-specific gene expression. Initial studies demonstrated that while the proximal promoter region (−168 bp to +21 bp) could direct low level expression in cultured cells, it could not support adipose expression in transgenic mice (1,2). We subsequently identified an enhancer extending from −5.4 kb to −4.9 kb that could direct adipose-specific expression of a chloramphenicol acetyl transferase (CAT) reporter gene in both cultured cells and transgenic mice (3,4). This enhancer has been used to target expression of a number of biologically interesting molecules to adipose tissue *in vivo*, including SV40 large T antigen (5), diptheria toxin A chain (6), and the insulin-sensitive glucose transporter GLUT4 (7).

The 518 bp aP2 enhancer has been systematically analysed, and multiple *cis*- and *trans*-acting factors important for its function in cultured 3T3-F442A adipocytes have been defined (8). The differentiation-dependent switch for this multicomponent enhancer appears to be provided by a novel cell-type-restricted nuclear factor termed ARF6, which binds at two sites within the enhancer. The ARF6 DNA recognition sequence resembles a type of hormone response element (HRE) known as DR-1 (direct repeat with one nucleotide spacer, 9). This motif has been shown to preferentially bind homodimers of the retinoid X receptor (10,11) and the liver-restricted transcription factor HNF4 (12), heterodimers of RXR and the orphan receptor COUP-TF (13), and heterodimers of RXR and the peroxisome proliferator-activated receptors (PPARs) (14). We have recently described a novel member of the peroxisome proliferator-activated receptor family, PPARγ2, that is expressed at high levels specifically in adipose tissue (9). PPARγ2 forms a heterodimeric complex with RXRα, and this complex can bind to and activate the aP2 enhancer. These observations suggested that PPARγ2 and RXRα were likely to be components of the endogenous ARF6 complex. However, it was still possible that additional and/or related proteins might contribute to the ARF6 binding activity present in adipocyte nuclear extracts.

The isolation of rare adipocyte proteins has not previously been technically feasible due to the unavailibility of a source of large quantities of differentiated adipocytes. We describe here a novel

*To whom correspondence should be addressed

+Present address: Department of Medicine, University of Chicago, Chicago, IL 60637, USA

system for the growth and differentation of an adipocyte cell line in suspension culture, and the purification of the transcription factor ARF6. We demonstrate that ARF6 is a heterodimeric complex of two nuclear hormone receptors, PPARγ and RXRα. These results suggest that PPARγ serves a unique function among PPAR family members as an important regulator of adipocyte-specific gene expression.

MATERIALS AND METHODS

Cell culture and nuclear extracts

HIB-1B brown adipocytes (5) were cultured in suspension in McCoy's SA medium containing 10% calf serum (Hyclone), 500μg/ml insulin and 1 nM triiodothyronine (Sigma). The cell density was kept above 5×10^4 cells/ml at all times. Cultures were diluted 1:1 with fresh media every 48 h and harvested in 8 L batches when the cells reached maximum density (approximately 3.0×10^5 cells/ml). A significant decrease in the ability of the culture to differentiate and accumulate lipid was observed after 10 passages. Nuclear extracts were prepared from 600 L of HIB-1B culture as described (15), except that the material was not dialyzed following salt extraction of the nuclei. The nuclear extraction buffer consisted of 0.6 M NaCl, 10 mM Hepes pH 7.9, 0.75 mM spermidine, 0.15 mM spermine, 1 mM EGTA, 0.1% NP-40, 1 mM DTT, and 20% glycerol.

Purification of ARF6

ARF6 binding activity was monitored by DNA mobility shift assay as described (9). Typically, HIB 1B adipocyte nuclear extract (20 mg protein/ml) was adjusted to 20 mM sodium phosphate pH 7.0 and loaded directly onto a BIO-GEL HTP hydroxylapatite column (BIO RAD) equilibrated with nuclear extract buffer containing 20 mM sodium phosphate pH 7.0. The HTP flow-through fraction was diluted to 120 mM NaCl in buffer A (20 mM Hepes pH 7.9, 1 mM EDTA, 0.1% NP-40, 1 mM DTT, 20% glycerol) and loaded onto a Whatman P-11 phosphocellulose column. ARF6 activity was eluted with 0.3 M NaCl in buffer A, diluted to 150 mM NaCl, and loaded onto a heparin−sepharose CL-6B column (Pharmacia). The heparin−sepharose column was eluted stepwise at 0.4 M and 0.5 M salt, with 90% of the detectable ARF6 activity being present in the 0.5 M fraction.

Sequence-specific DNA sepharose CL-4B affinity resins were constructed by the method of Kadonaga *et al.* (16) using wild type (5′-GATCTGTGA ACTCTGATCCAGTAAG-3′) and mutant (5′-GATCTGTGAACACAGATGGAG TAAG-3′) synthetic double-stranded ARE7 oligonucleotides. The 0.5 M Heparin−sepharose fraction was diluted to 0.2 M NaCl with buffer A, adjusted to 100 mg/ml sonicated salmon sperm DNA (Sigma), and incubated overnight at 4°C with 0.5 ml of the ARE7 affinity resin. The following morning, the affinity resin was poured into a column, washed extensively with 0.2 M NaCl, and eluted stepwise with 0.3 M and 0.5 M NaCl. All detectable ARF6 activity was present in the 0.5 M fraction. The 0.5 M fraction was diluted to 0.2 M NaCl and subjected to a second round of purification with the ARE7 affinity resin. Samples of the purified material were electrophoresed through 8.0% polyacrylamide gels and stained with silver nitrate using the Stratagene Silver Stain Kit. Multiple preparations of affintity purified ARF6 were pooled for tryptic digestion. The final yield of the purification was approximately 5μg ARF6 from 600 L HIB-1B suspension culture.

Protein analysis

The most highly purified ARF6 preparations were precipitated, fractionated by SDS polyacrylamide gel electrophoresis, transferred to nitrorocellulose and visualized by Ponseau S staining. Bands of interest were excised and processed for internal amino acid sequence analysis as described (17), with modifications. *In situ* proteolytic cleavage was carried out using 1μg trypsin (Promega) in 25μl 100 mM NH_4HCO_3 (supplemented with 10% acetonitrile and 3% Tween-80) at 37°C for 3 h. The resulting peptide mixture was reduced with 0.1%β-mercaptoethanol (BioRad), S-alkylated with 0.3% 4-vinyl pyridine (Aldrich), and fractionated by reversed phase HPLC. An enzyme blank was run on an equally-sized strip of nitrocellulose.

HPLC solvents and system configuration were as described (18), except that a 2.1 mm Vydac C4 (214TP54) column was used with gradient elution at a flow rate of 100μl/min. Tryptophan-containing peptides were identified by manual ratio analysis of absorbances at 2α and 277 nm, monitored in real time using an Applied Biosystems model 1000S diode-array detector (19).

Peptides were analyzed by a combination of automated Edman degradation and matrix-assisted laser-desorption mass spectrometery (MALDI-TOF); details about this combined approach can be found elsewhere (18,20). Mass analysis (on 2% aliquots) was carried out using a LaserTec Research instrument (Vestec); the matrix wasα-cyano-4-hydroxy cinnamic acid (Linear Sci). Each sample was analyzed twice, in the presence and absence of calibrant (25 femtomoles APID), as described (20). Chemical sequencing (on 95% of the sample) was performed using a model 477A instrument (Applied Biosystems) with 'on-line' analysis (120A HPLC system with a 2.1×220 mm PTH C18 column). Instruments and procedures were optimized for femtomole-level phenyl thiohydantoin amino acid analysis as described (21).

Peptide sequences were compared to entries in various sequence databases using the National Cancer Center for Biotechnology Information (NCBI) BLAST program. Average isotopic masses of predicted peptides were summed from the published sequences using ProComp version 1.2 software (obtained from P.C. Andrews, University of Michigan, Ann Arbor, MI.)

Oligonucleotides and DNA binding assays

The sequences of the double-stranded oligonucleotides used were as follows: (only one strand shown): PCK2, 5′-GATCCGAG-ACCTTTATCCCAGTTGT-3′; M5, 5′-GATCTGTGAAGT-CTGATCCAGTAAG-3′. The ARE2, ARE6, and ARE7 oligonucleotides were described previously (8,9) . DNA mobility shift assays were performed as in (9). When highly purified ARF6 samples were assayed, BSA was added to the binding reaction at a final concentration of 200μg/ml. 3T3-F442A preadipocyte and adipocyte nuclear extracts were prepared as described (15), except that extracts were not dialyzed but added directly to the binding reaction. Methylation interference assays were carried out under standard conditions using methylated ARE7 probe as described (22).

RNA analysis

Total RNA was isolated from cultured cells and tissues of adult mice by guanidine isothiocyanate extraction (23). S1 nuclease

analysis was performed as decribed (24) using an end-labeled 520 bp *StuI* restriction fragment derived from mPPARγ2 SPORT (9) as probe. This fragment contains 110 bp of vector sequence followed by the first 410 bp of of the mPPARγ2 cDNA.

RESULTS

In order to identify the proteins which comprise the ARF6 complex, we purified this DNA binding activity from adipocyte nuclear extracts. Purification of an adipocyte transcription factor required the generation of large quantities of differentiated adipocytes. We found that the brown fat tumor (hibernoma) cell line HIB-1B recently established in our laboratory (5) could be readily grown and differentiated in suspension culture. When cultured in suspension in the presence of triiodothyronine and insulin, HIB-1B cells accumulate lipid and express the characteristic repertoire of adipocyte genes (25–27), without losing the ability to undergo cell division (data not shown, see Materials and Methods for details of cell culture). HIB-1B cells were derived from the brown adipocyte tumor of a transgenic mouse expressing SV40 large T antigen under the control of the aP2 enhancer. We therefore expected these cells to express significant levels of the putative key regulator of this enhancer, ARF6. DNA mobility shift assays were performed using a radiolabeled double-stranded oligonucleotide containing the ARE7 site from the aP2 enhancer (8). As shown in Figure 1, HIB-1B cells (lane 3) appear to contain the same differentiation-dependent nuclear factor previously identified in 3T3-F442A adipocytes, ARF6 (lane 2). A significant increase in ARF6 binding activity is observed when HIB-1B cells are induced to undergo terminal

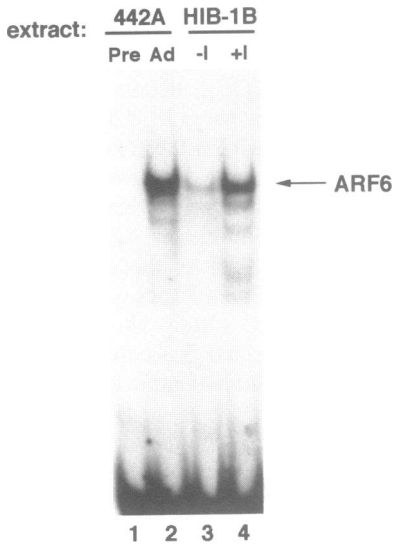

Figure 1. HIB-1B brown adipocytes grown in suspension express ARF6 binding activity. Double-stranded ^{32}P-labeled ARE7 oligonucleotide was used as probe in a DNA mobility shift assay with 3T3-F442A preadipocyte nuclear extract, 3T3-F442A adipocyte nuclear extract, uninduced HIB-1B nuclear extract, or induced HIB-1B nuclear extract. DNA-protein complexes were resolved from free DNA on a 5 % polyacrylamide gel. The gel was dried and exposed to film for 2 h at −70°C.

Table 1. Purification of ARF6 from HIB-1B nuclear extract

Fraction	total protein (mg)	specific activity (U/mg)[a]	fold purification	cumulative yield %
NE	2,400	16	—	100
HA FT	792	51	3.1	88
PC	75	384	23.6	64
Hep Seph	20	864	54.3	38
Affinity II	0.007	207,142	13,018.0	3.2

[a]One unit is arbitrarily defined as the activity required to shift 5 fmol of ARE7 oligonucleotide probe under standard conditions.

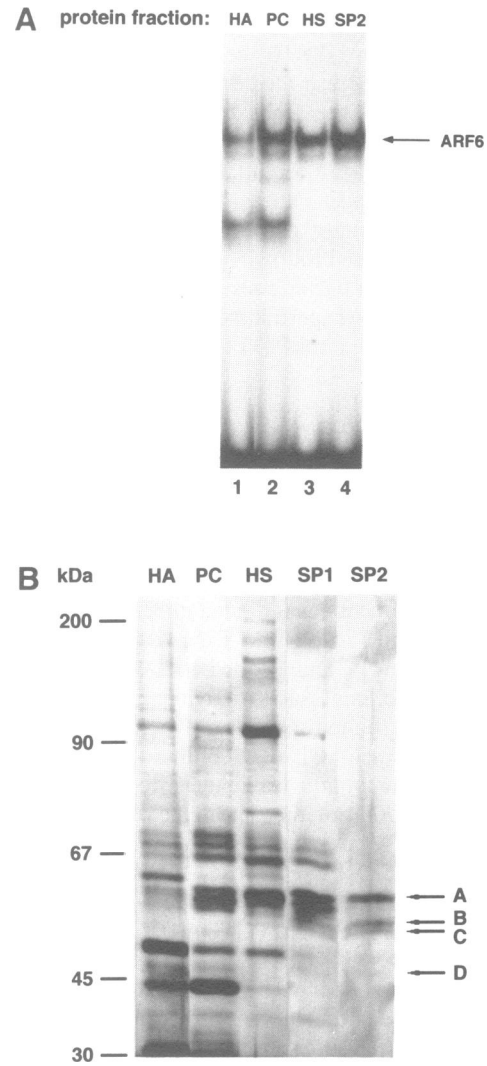

Figure 2. (A) DNA binding activity of purified ARF6 protein fractions. Double-stranded ^{32}P-labeled ARE7 oligonucleotide was used as probe in a DNA mobility shift assay with 5μg of the hydroxylapatite flow-through fraction (HA), 1μg of the phosphocellulose 0.3 M fraction (PC), 0.3μg of the heparin–sepharose 0.5 M fraction (HS), and 0.002μg of the secondary ARE7 affinity fraction (SP2). DNA–protein complexes were resolved from free DNA on a 5 % polyacrylamide gel. The gel was dried and exposed to film for 1 h at −70°C. (**B**) Purified ARF6 preparations contain four major polypeptide species. Samples of the hydroxylapatite flow-through fraction (HA), the phosphocellulose 0.3 M fraction (PC), the heparin–sepharose 0.5 M fraction (HS), and 0.1μg of the secondary ARE7 affinity fraction (SP2) were electrophoresed through an 8.5% SDS–polyacrylamide gel and stained with silver as described in Experimental Procedures

differentiation by treatment with insulin and triiodothyronine (lane 4). This increase parallels that observed for aP2 mRNA levels (5).

The ARF6 purification protocol is described in detail in Materials and Methods and a sample purification is summarized in Table 1. Throughout the procedure, ARF6 activity was monitored by DNA mobility shift assay. We found that dialysis of the crude nuclear extract resulted in a profound loss of ARF6 binding activity. Hydroxylapatite chromotography was employed as the first step to obviate the need for dialysis and to remove contaminating nucleic acids. HIB-1B nuclear extract was adjusted to 20 mM sodium phosphate pH 7.0 and applied directly to a BIO-GEL HTP hydroxylapatite (HA) column equilibrated with extract buffer plus 20 mM sodium phosphate pH 7.0. All detectable ARF6 activity was present in the flow through fraction, and approximately 70% of the total protein and all of the contaminating nucleic acid was retained on the column. The hydroxylapatite flow-through fraction was adjusted to 0.1 M NaCl by dilution with standard buffer and further purified by successive phosphocellulose (PC) and heparin–sepharose (HS) chromatography, with ARF6 activity eluting at 0.3 M and 0.5 M NaCl respectively.

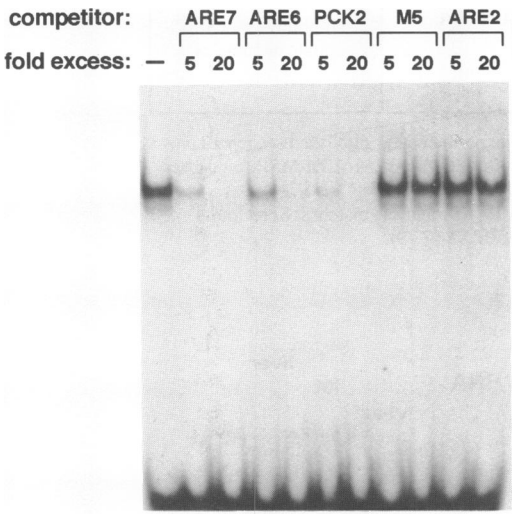

Figure 3. DNA binding specificity of purified ARF6. Double-stranded ^{32}P-labeled ARE7 oligonucleotide was used as probe in a DNA mobility shift assay with purified ARF6 protein. Double-stranded competitor oligonucleotides were added in the molar excesses indicated. DNA–protein complexes were resolved from free DNA on a 5 % polyacrylamide gel. The gel was dried and exposed to film for 1 h at −70°C.

The most significant step in the procedure was sequence-specific DNA affinity chromatography, which was performed on an ARE7 oligonucleotide affinity resin (See Materials and Methods). After two consecutive passes over the ARE7 affinity column, the active fractions contained four major polypeptide species with approximate molecular masses 55 kDa, 52 kDa, 51 kDa and 48 kDa (Figure 2B). These polypeptides will be referred to subsequently as A, B, C, and D respectively. When the heparin–sepharose fraction is subjected to two rounds of chromatography with a mutant ARE7 oligonucleotide column, these four polypeptides are absent from the equivalent fractions, indicating that all four are sequence-specific DNA binding proteins (data not shown). Although additional polypeptides were present in the purified fractions, only these four polypeptides bound specifically to the wild-type but not the mutant affinity column. The ARE7 binding activities in the phosphocellulose (PC), heparin-sepharose (HS), and secondary ARE7 affinity fractions (SP2) migrate with precisely the same mobility as ARF6 from adipocyte nuclear extract (Figure 2A).

We performed several experiments to confirm that the binding specificity of the purified material was identical to that of the ARF6 activity in crude nuclear extract. First, we assayed the

Table 2. Summary of peptide sequencing and mass spectrometric analysis of tryptic peptides derived from samples A and D

Polypeptide A			Polypeptide D		RXRα sequence[b]	
peptide	sequence	*m/z*	peptide	*m/z*	position	[MH+]
T56	HFLPLDFSTQVN...	2404.6	not found[a]		5-26	2405.64
T74	GSMAVPSLHPSLGPGIGSPL...	9632.6	not found[a]		27-123	9579.09
T64	not determined	1235.5	T54	1235.8	280-289	1235.47
T67	IPHFSELPLDDQVILLR ||	2006.1	T58	2006.4	291-307	2006.36
T66	not determined	1601.6	T56	1602.2	308-321	1601.81
T83	LIGDTPIDTFLMEMLEAPHQ...	2444.0	T74	2443.9	446-467	2444.84

Symbols:[MH+], theoretical average isotopic mass of peptide (from experimental or published sequence) plus one proton. *m/z*, experimental mass determined by laser-desorption mass spectrometry (MALDI-MS) ||, indicates C-terminus of peptide. ..., no more interpretable signals but unlikely to be C-terminus of pepetide. [a]All fractions around corresponding elution position were screened by MS and no matches were found.
[b]Corresponding amino acid position in the open reading frame of RXRα (29).

Table 3. Summary of peptide sequencing and mass spectrometric analysis of tryptic peptides derived from samples B and C.

Polypeptide B			Polypeptide C		mPPARγ2 sequence[b]	
peptide	sequence	*m/z*	peptide	*m/z*	position	[MH+]
T52	TTVDFSSISAPHYEDIPFTR ‖	2284.0	T53	2284.5	66-85	2284.50
T22	ASGFHYGVHACEGCK ‖	1775.9	T22	1775.9	146-160	1776.76
T32	PGHLQEGFGCVVTNR ‖	1720.4	not found[a]		no homology with known sequences	
T66	FDQLFDDESDPFEVLK ‖	1944.8	not found[a]			

Symbols: [MH+], theoretical average isotopic mass of peptide (from experimental or published sequence) plus one proton. *m/z*, experimental mass determined by laser-desorption mass spectrometry (MALDI-MS) ||, indicates C-terminus of peptide.
[a]All fractions around corresponding elution position were screened by MS and no matches were found.
[c]Corresponding amino acid position in the open reading frame of mPPARγ2 (9).

affinity of the purified material for synthetic oligonucleotides containing three different ARF6 binding sequences: the ARE6 and ARE7 sites from the aP2 enhancer (8), and the PCK2 site from the PEPCK enhancer (28). Oligonucleotides containing mutant ARE7 and non-specific sequences were assayed in parallel. As shown in Figure 3, the purified material binds well to ARE7 and PCK2, and with slightly lower affinity to ARE6. It does not bind to a mutant ARE7 site containing a single base mutation (see Materials and Methods) or the ARE2 site from the aP2 enhancer (8). Identical binding specificity has been described previously for the ARF6 activity in crude extracts (8,9). Second, we compared the methylation interference footprint of the purified material on the ARE7 site with that determined previously for ARF6 in crude extract (9). Identical guanosine residues are identified as points of DNA−protein interaction when the purified protein is used in this assay (not shown).

The most highly purified material from multiple preparations was pooled, electrophoresed through an 8.0% SDS−polyacrylamide gel, and transferred to a nitrocellulose membrane. After visualization by Ponseau S staining, the 55 kDa, 52 kDa, 51 kDa and 48 kDa polypeptides were individually isolated and subjected to tryptic digestion *in situ*. Tryptic peptides derived from each polypeptide were fractionated by narrow bore reverse phase HPLC, analyzed by mass spectrometry, and sequenced as described in Materials and Methods. The data obtained from these studies are summarized in Tables 2 and 3. Six peptides from the 55 kDa polypeptide (A) were identified as fragments of the retinoid X receptor α (RXR α, 29); four of these peptides were also identified in the 48 kDa polypeptide (D). Two peptides corresponding to the N-terminus of RXRα (T56 and T74) were identified in the 55 kDa sample but not the 48 kDa sample, suggesting that the smaller polypeptide is an N-terminally truncated form of RXRα (Table 2).

Analysis of the 52 kD and 51 kDa polypeptides (B and C) is summarized in Table 3. Peptide sequencing and mass spectral analysis identified two polypeptides present in both samples as fragments of the murine peroxisome proliferator-activated receptorγ (PPARγ, 9, 30, 31, 32). From these two peptides it could not be determined which of the known PPARγ isoforms

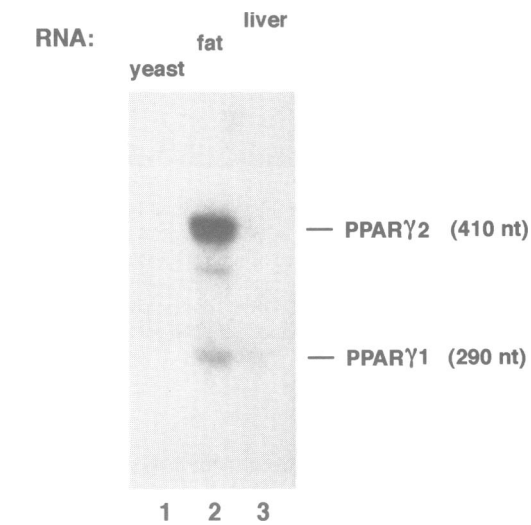

Figure 4. Expression of PPARγ1 and PPARγ2 isoforms in adipose tissue and liver. S1 nuclease analysis was performed using 10 mg of RNA from yeast (control) and adipose tissue and liver of adult mice. A 520 bp ^{32}P end-labeled DNA fragment containing the first 410 bp of the PPARγ2 cDNA was used as probe. Reaction products were resolved on an 8% polyacrylamide sequencing gel. The gel was dried and exposed to film for 12 h at −70°C.

was present in these samples. HPLC fractionation indicated that while the majority of tryptic peptides derived from the 52 kD sample were present in the 51 kDa sample (data not shown), the 52 kD sample also contained several unique peptides. Two of these unique peptides were sequenced (T32 and T66). No homology with any known protein was observed for these sequences. It is not yet clear whether these peptides derive from a novel isoform of PPARγ or a contaminating protein present in the 52 kDa sample. The two RXRα species (polypeptides A and D) appear to be present in an approximate molar ratio of 1:1 with the two PPARγ species (polypeptides B and C) in the purified ARF6 preparations. PPARs have been demonstrated previously to bind DNA only in conjunction with RXRs (14,33).

We conclude from these data that the adipocyte ARF6 complex is a heterodimer of PPARγ and RXRα. These results establish a role for specific members of the PPAR and RXR nuclear receptor subfamilies in the regulation of adipocyte-specific gene expression.

Since two different isoforms of PPARγ (γ1 and γ2) have been described (9,31,32), S1 nuclease analysis was performed to quantitate the relative abundance of these isoforms in adipose tissue. A 540 nt end-labeled probe derived from the 5′ end of the PPARγ2 cDNA (see Materials and Methods) is degraded to a 410 nt fragment when hybridized to the PPARγ2 mRNA, and to a 290 nt fragment when hybridized to the PPARγ1 mRNA. Figure 4 demonstrates that PPARγ2 mRNA is at least ten times more abundant than PPARγ1 mRNA in adipose tissue (lane 2). The very low level of PPAR mRNA expression observed in liver encodes predominantly the PPARγ1 isoform (lane 3).

DISCUSSION

Prior to this work an efficient method of generating large quantities of cultured adipocytes had not been described. The purification of adipocyte-specific proteins has therefore not previously been technically feasible. The HIB-1B suspension culture system described here represents a valuable resource for the isolation of rare biological molecules from adipose cells. HIB-1B cells are a transformed, semi-differentiated brown adipocyte cell line that can be induced to undergo terminal differentiation when grown in suspension. Unlike the white adipocyte cell lines 3T3-F442A and 3T3-L1, HIB-1B cells accumulate lipid and express terminal markers of adipocyte differentiation without losing the capacity to undergo cell division.

We have used the HIB-1B suspension culture system to purify the adipocyte transcription factor ARF6. Several lines of evidence suggest that this factor is an important regulator of adipogenic gene expression. While the proximal promoters of a number of adipocyte genes have been analyzed in cultured cells, the aP2 and PEPCK enhancers are the only DNA sequences thus far identified that are capable of directing adipose tissue-specific expression *in vivo* (3,34,35). ARF6 binding sites have been shown to be of critical importance to the activity of both the aP2 and PEPCK adipocyte-specific enhancers (8,28). Moreover, ARF6 binding activity is detected only in nuclear extract derived from differentiated adipocytes, and multiple copies of ARF6 binding sequences are sufficient to direct cell type-specific expression of a reporter gene in cultured cells (8).

Chemical sequencing and mass spectral analysis of tryptic peptides derived from affinity purified ARF6 revealed that this complex is composed of the murine PPARγ and RXRα. This represents the first definitive demonstration that members of the PPAR and RXR nuclear hormone receptor families are involved in tissue-specific, differentiation-linked gene expression. PPARs are a subfamily of nuclear hormone receptors first identified by virtue of their ability to be activated by agents such as the clofibrate hypolipidemic drugs, which induce peroxisome proliferation in rodent liver (36). Multiple PPAR family members have now been described in both mouse (α,γ,δ, and NUC-1 9, 30, 31, 32, 36, 37) and Xenopus (α,β,γ, 38). Each family member is highly conserved between species, for example, mPPARγ is more similar to xPPARγ than it is to mPPARα (31,38). This suggests that the different PPARs are likely to have distinct physiologic roles. This possibility is supported by the observation that the murine PPARs have distinct tissue distributions: PPARγ is extremely abundant and fat-specific (9), PPARα is expressed predominantly in liver, heart, and kidney (36), and NUC-1 is expressed in many tissues (39). While PPARα and NUC-1 have been reported to be expressed at low levels in adipose tissue (40), our data indicate that they do not contribute significantly to the adipocyte ARF6 complex. The identification of PPARγ in the ARF6 complex, together with the abundance and tissue-specificity of PPARγ expression, suggests that the biological role of this particular PPAR family member is that of an important regulator of adipocyte-specific gene expression. Of the known PPARγ isoforms, PPARγ2 is the predominant form expressed in adipose tissue.

The endogenous ligand for the PPARs, if one exists, remains unknown. Although a number of lipid-like molecules, including the clofibrate hypolipidemic drugs, arachadonate analogs and polyunsaturated fatty acids can activate PPARs in experimental systems, none of these have been shown to bind the receptors directly (14,33,41). A number of synthetic PPAR activators, including 5,8,11,14-eicosatetraynoic acid (ETYA), pirixinic acid, and clofibrate-related compounds have been shown to promote the differentiation of certain preadipocyte cell lines (40,42,43). An intriguing possibility raised by our findings is that one or more lipids derived from dietary intake might directly regulate adipocyte gene expression through their interaction with the adipocyte-specific receptor PPARγ2. Identification of the endogenous adipocyte ligand for PPARγ2 will be a primary goal of future research. It might be eventually be possible to pharmacologically control adipocyte gene expression and/or differentiation with specific activators or inhibitors of PPARγ2.

Although retinoids function in most cell types as promoters of differentiation, they are known to inhibit the differentiation of cultured adipocytes (44). The identificaton of RXRα in a key adipocyte regulatory complex suggests a possible mechanism for this effect. 9-cis retinoic acid has been shown to preferentially stabilize the formation of RXR homodimers (11). In adipocytes, 9-cis retinoic acid might be expected to induce RXRα homodimerization at the expense of the PPARγ2/RXRα heterodimer. This may compromise the expression of differentiation-linked genes controlled by the PPARγ2/RXRα complex, leading to a blockage of terminal differentiation. Future studies will endeavor to determine whether experimental alteration of PPARγ2 and RXRα expression can modulate the adipogenic program.

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