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AdipoQ Is a Novel Adipose-specific Gene Dysregulated in Obesity*

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Adipose differentiation is accompanied by changes in cellular morphology, a dramatic accumulation of intracellular lipid and activation of a specific program of gene expression. Using an mRNA differential display technique, we have isolated a novel adipose cDNA, termed adipoQ. The adipoQ cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a collagenous region (Gly-X-Y repeats), and a globular domain. The globular domain of adipoQ shares significant homology with subunits of complement factor C1q, collagen α1(X), and the brain-specific factor cerebellin. The expression of adipoQ is highly specific to adipose tissue in both mouse and rat. Expression of adipoQ is observed exclusively in mature fat cells as the stromal-vascular fraction of fat tissue does not contain adipoQ mRNA. In cultured 3T3-F442A and 3T3-L1 preadipocytes, hormone-induced differentiation dramatically increases the level of expression for adipoQ. Furthermore, the expression of adipoQ mRNA is significantly reduced in the adipose tissues from obese mice and humans. Whereas the biological function of this polypeptide is presently unknown, the tissue-specific expression of a putative secreted protein suggests that this factor may function as a novel signaling molecule for adipose tissue.

Adipose tissue is highly specialized to play important roles in energy storage, fatty acid metabolism, and glucose homeostasis (1, 2). Adipocytes synthesize and store triglyceride in periods of nutritional abundance and mobilize the lipids in response to fasting (2, 3). Fat tissue is also involved in regulating blood glucose levels through the expression of the insulin responsive glucose transporter, Glu4 (4, 5). Fat and muscle, in fact, constitute the two major sites for insulin-regulated glucose uptake.

At a molecular level, many genes involved in lipid metabolism and glucose homeostasis are prominently expressed in fat (1). These include fatty acid synthase (6), the fatty acid binding protein aP2 (7, 8), lipoprotein lipase (9), phosphoenolpyruvate carboxykinase (10), malic enzyme (11), glyceraldehyde-3-phosphate dehydrogenase (12), and Glut4 (4). Receptors for lipogenic or lipolytic hormones such as insulin (13, 14), insulin-like growth factor 1 (15), and adrenergic compounds (16, 17) are also expressed in adipocytes. In addition to these genes that clearly participate in the metabolic functions of adipose tissue, a group of genes that function in extracellular signaling have also been identified in fat. A prototype of these molecules is insulin-like growth factor 1, which is expressed in many tissues during development and plays an important role in cell proliferation (18). In adipocytes, however, insulin-like growth factor 1 is found to stimulate cell differentiation (19). More interestingly, insulin-like growth factor 1 is synthesized by preadipocytes in response to growth hormone stimulation (20), thus potentially functioning in an autocrine or paracrine fashion to promote adipogenesis during development. Another signaling molecule from adipose tissue is TNF-α.[1] TNF-α is secreted from fat, especially in obesity, and acts in an autocrine or paracrine manner to interfere with insulin action in fat and muscle (21, 22). The recent cloning and characterization of the ob gene product has further illustrated that adipose tissue secretes signaling molecules that function in an endocrine fashion (23). The *ob* gene product (leptin) is secreted from fat into the circulation and acts to regulate body weight, perhaps via a putative receptor in the cerebroventricular region of the brain (15, 23, 24). Hence, molecules secreted from adipose tissue are capable of modulating diverse functions in fat and other tissues, thus representing a new facet of adipose tissue physiology.

In this study, we have used mRNA differential display to clone a novel adipose gene termed adipoQ. Sequence analysis suggests that adipoQ is a secreted protein that shares significant homology to subunits of complement factor C1q and contains a collagenous structure at the NH_2 terminus and a globular domain at the COOH terminus. The expression of this novel gene is highly regulated during the adipose differentiation process and is expressed predominantly in adipose tissue *in vivo*. Moreover, a significant down-regulation in adipoQ mRNA was observed in fat tissues from obese mice and humans. Our results provide a potentially valuable new molecular tool to explore the physiology of adipose tissue in normal and pathological states.

EXPERIMENTAL PROCEDURES

Materials—DMEM, calf serum, and fortified calf serum were from Hyclone, Inc. Insulin, dexamethasone, and isobutylmethylxanthine were purchased from Sigma, Inc. Reverse transcriptase (Moloney murine leukemia virus), Klenow fragment and T4 kinase were obtained from BRL, Inc. Isotopes including [^{32}P]dCTP (6000 Ci/mmol) and ^{35}S-dATP (1300 Ci/mmol) were from DuPont NEN. GenAMP kit containing *Taq* DNA polymerase was purchased from Perkin-Elmer.

Cell Lines and Cell Culture—Murine fibroblastic 3T3-C2 cells and 3T3-F442A and 3T3-L1 preadipocytes were cultured as described (7, 25). Induction of adipocyte differentiation was performed essentially as described (26). Briefly, differentiation was initiated by administration of insulin at 5 μg/ml at confluence for 3T3-F442A cells and dexamethasone (1 μM), isobutylmethylxanthine (0.25 mM), and insulin (5 μg/ml) for 3T3-L1 cells. For 3T3-L1 cells, cells were treated with dexamethasone/isobutylmethylxanthine/insulin mix for 48 h and then were refed by DMEM medium containing 10% fetal calf serum and 5 μg/ml insulin.

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[1] The abbreviations used are: TNF-α, tumor necrosis factor α; DMEM, Dulbecco's modified Eagle's medium; PCR, polymerase chain reaction.

Using this protocol, more than 90% of the cells in both cell lines acquire an adipocyte morphology 5–7 days after the initiation of differentiation. Culture medium was routinely changed every 2 days, and adipocyte differentiation was examined visually under the microscope.

mRNA Differential Display—mRNA differential display was performed essentially as described (27, 28). Briefly, total cellular RNA was isolated from 3T3-C2, 3T3-F442A preadipocytes, and differentiated 3T3-F442A adipocytes using the guanidine isothiocyanate extraction (29). 50 μg of total RNA was then treated with 20 units of RNase free-DNase (BRL, Inc.). Subsequently 0.2 μg of treated RNA was used in a reverse transcription reaction using each of the four 1-base pair-anchored 3′ oligo(dT) primers (30) and 300 units of Mo-MLV reverse transcriptase (BRL) in 20 μl volume as recommended by the manufacturer. 2 μl of the reverse transcribed cDNA was used for each PCR reaction. PCR reaction was performed using the same 1-base pair-anchored 3′ oligo(dT) primer and 10 5′ arbitrary oligos of 10 nucleotides in length. The sequence of the 5′ arbitrary oligonucleotide that gave DD1 PCR product (see text) is 5′-AGTCATACAT-3′. The 50-μl PCR reaction contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 2 uM dNTP (except dATP), 1 μl of α-^{35}S-dATP (1300 Ci/mmol), 2 uM of 5′ arbitrary oligo and 3′ anchored dT oligo, and 0.2 μl of *Taq* polymerase. Parameters for PCR were 30 cycles of denaturing at 95 °C for 30 seconds, annealing at 40 °C for 1 min, and extension at 72 °C for 30 seconds. 5 μl of the PCR reaction mixture was loaded on a 8% sequence gel, and differentially amplified PCR fragments were visualized by exposing the dried sequencing gel to x-ray film. Candidate PCR products were excised from the sequencing gel, and the DNA was eluted from the gel slices by boiling the gel slice in TE (10 mM Tris, pH 7.5, 1 mM EDTA) buffer for 10 min. The eluted DNA fragment was re-amplified by using the same primer pair and subsequently cloned into the TA cloning vector (Invitrogen, Inc.).

Library Screening, cDNA Cloning, and Sequencing—The cDNA library screening, restriction fragment analysis, subcloning, and sequencing analysis were performed as described (31). An adipocyte-specific λZAP II cDNA library was custom made by Stratagene Inc. as described (26). GenBank data base searches were performed using the Eugene program at the computer service in Dana-Farber Cancer Institute, and further homology searches were performed using Blast and Autosearch programs. Homology alignments were completed using the Pileup program from the Genetics Computer Group sequence analysis package (Madison, Wisconsin).

In Vitro Translation of AdipoQ cDNA—*In vitro* transcription and translation was performed using a TNT *in vitro* translation system from Promega, Inc. according to the manufacturer's instructions. [^{35}S]methionine (800 Ci/mmol, DuPont NEN) was used to label the translated protein and visualized on 12% SDS-polyacrylamide gel after fluorography. Molecular markers were from Amersham Corp. (rainbow markers).

Expression of Flag-tagged AdipoQ—The coding sequence of adipoQ was subcloned into pSV-sport eukaryotic expression vector (BRL) using oligonucleotides 5′-GAATTCGGGATGCTACTGTTGCAAGCT-3′ and 5′-CTCTTCCATGATACCAACGACTACAAGGACGACGATGACAAG-TGAGAATTC-3′. The flag-epitope (DYKDDDDK, Kodak Scientific Imaging, Inc) was incorporated into the COOH terminus. The NIH-3T3 fibroblasts were transient transfected with pSV-sport-flag-adipoQ as described (31). 24 h after transfection, cells were washed with phosphate-buffered saline, and DMEM medium with no serum was added. DMEM medium was collected after 24 h, and cells were lysed with RIPA buffer as described (31). Antibody against Flag-epitope (M2 monoclonal antibody, Kodak) was used to immunoblot the proteins separated on SDS-polyacrylamide gel electrophoresis.

RNA Isolation and Northern Blot Analysis—Total RNA was isolated from both 3T3-F442A and 3T3-L1 cell lines as well as from various mouse, rat, and human tissues as described (29, 31). 10 μg of RNA was denatured in formamide and formaldehyde at 55 °C and separated in formaldehyde-containing gels as described (31). RNA was blotted onto Hybond nylon membranes, and the nylon membranes were baked, hybridized, and washed as directed by the manufacturer. cDNA probes were radiolabeled to specific activities of at least 10^9 cpm/μg with [α-^{32}P]dCTP (6000 Ci/mmol) using the random priming method (32).

Fractionation of Rat Fat Pad into Stromal-vascular and Fat Cells—Fractionation of rat fat fads was performed as described (33). Briefly, epididymal fat deposits were removed and transferred into Petri dishes containing the DMEM supplemented with 10% fetal calf serum and antibiotics (penicillin 100 μg/ml, streptomycin 10 μg/ml). The fat pads were minced by surgical scissors and digested with collagenase (5 mg/ml) for 45 min at 37 °C under agitation. The resulting cell suspension was filtered through a 100-μm nylon filter and centrifuged at 400 × *g* for 5 min. The floating mature adipocytes were washed and centrifuged again. The pelleted fractions were collected and combined. Floating adipocytes and the pelleted stromal-vascular fraction were lysed with guanidine isothiocyanate, and total RNA was isolated as described (29).

RESULTS

Identification of AdipoQ cDNA—To identify novel genes that are differentially expressed during adipose differentiation, we employed an mRNA differential display technique (27, 34). RNA samples were prepared from 3T3-C2 cells (C), a fibroblastic cell line unable to differentiate into adipocytes, and a similarly derived preadipocyte cell line, 3T3-F442A cells before (P) and after (A) differentiation (25, 35). The RNA was used to synthesize the corresponding cDNA, which was subsequently used for differential display PCR reaction using 10 different arbitrary 5′ primers (see "Experimental Procedures"). A number of candidate α-^{35}S-dATP-labeled PCR products visualized in sequencing gels were expressed preferentially in mature adipocytes (data not shown), and we focused on one such product, DD1 (Fig. 1*A*). A partial cDNA clone for DD1 was obtained by PCR re-amplification (see "Experimental Procedures") and sequenced. No significant sequence homology with any other genes in GenBank was apparent from this 200-base pair fragment. However, putative polyadenylation signals were present in this short nucleotide sequence. Northern analysis using this cDNA fragment revealed a mRNA expressed predominantly in differentiated fat cells (Fig. 1*B*). These data suggested that this cDNA fragment reflected a genuine mRNA species that was differentially regulated. A full-length clone of DD1 was subsequently obtained by screening a λZAP II cDNA adipocyte library with the partial cDNA clone. Sequence analysis revealed a single open reading frame in the full-length cDNA clone (Fig. 2*A*).

Analysis of the putative protein sequence identified a hydrophobic leader from amino acid residues 2 to 17, presumably representing a signal peptide. A region of collagenous repeats (Gly-X-Y) was present from amino acids 45 to 110, with 22 individual Gly-X-Y repeats. Comparisons with genes in GenBank identified several regions of homology to the subunits (A, B, and C chains) of complement factor C1q (36, 37), a tissue-specific collagen α1(X) (38), and a brain-specific protein cerebellin (39). The identity with the C1q chains is approximately 31% in the globular COOH-terminal region (Fig. 2*B*), with the homology localized primarily in two segments of uncharged, hydrophobic regions (Fig. 2*C*). In addition, adipoQ and C1q A, B, and C chains have a similar size of 240–250 amino acids (Fig. 2*B*). The number of Gly-X-Y repeats is similar as well, with 22 such repeats for adipoQ and 26–29 for the C1q chains. The similarity of this protein to collagen α1(X) and cerebellin is found mainly at the COOH-terminal globular domain (Fig. 2*C*) with 38 and 25% identity over a 130-amino acid region. Collagen α1(X), however, encodes a much larger protein (680 amino acids) with a long collagenous segment (154 Gly-X-Y repeats). Cerebellin, on the other hand, is a smaller polypeptide with 193 amino acid residues and does not contain a collagenous domain (39). Because of the similarity between this novel protein and all three components of C1q molecules in size, domain structure and overall homology, we termed this novel protein adipoQ.

AdipoQ is clearly a distinct member of a proteins family characterized by a collagenous helical structure at the NH_2 terminus, and a globular domain at the COOH terminus (40). In addition to C1q A, B, and C chains (36, 37) and collagen α1(X) (38), this protein family includes lung surfactant proteins SP-A and SP-D (41), mannan binding protein (42), and the scavenger receptor and its homolog (43, 44). These proteins often homo- or hetero-oligomerize via the collagenous struc-

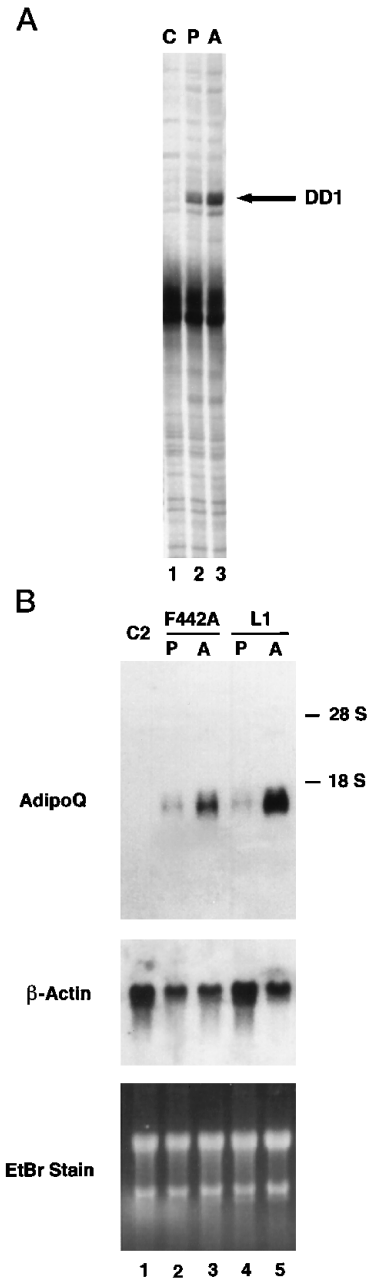

FIG. 1. **Identification of a novel adipocyte-specific mRNA.** *A*, mRNA differential display reactions were performed as described under "Experimental Procedures." ^{35}S-Labeled PCR products were visualized by autoradiography. *Lanes 1*, *2*, and *3* represent samples from 3T3-C2 (*C*), 3T3-F442A preadipocytes (*P*), and differentiated 3T3-F442A adipocytes (*A*). DD1 indicates a candidate PCR product that is differentially regulated. *B*, Northern analysis using DD1 as a probe. 10 μg of RNA from 3T3-C2 fibroblasts (*lane 1*, *C2*), 3T3-F442A preadipocytes (*lane 2*, *P*) and adipocytes (*lane 3*, *A*), and 3T3-L1 preadipocytes (*lane 4*, *P*) and adipocytes (*lane 5*, *A*) was analyzed. Ethidium bromide (*EtBr*) staining and hybridization to a β-actin probe were used to normalize RNA loading.

tures. The presence of a collagenous domain in adipoQ suggests that this protein is likely to form oligomeric structures by itself or with other proteins.

Although the COOH-terminal region of adipoQ shares significant similarity with the C1q chains, it also has some notable differences. For example, a cysteine (marked ‡ in Fig. 2*B*) in the globular region of C1q B (residue 194) and C (residue 198) chains is known to form disulfide bonds with activator molecules, *e.g.* IgG (45). In adipoQ sequence, this cysteine is not conserved and is replaced by an aspartate residue. Another conserved cysteine (residue 180 for C1q C chain, marked # in Fig. 2*B*) that is important for the formation of disulfide bonds and the stabilization of triplex strands in the collagenous domain (37, 46) is also altered in adipoQ. In addition, an interruption (marked * in Fig. 2*B*) in a collagenous motif found in C1q A (residue 61) and C (residue 65) chains is absent in adipoQ. These interruptions have been shown to be conserved between human and mice and result in a bend in the collagen triplex formation that can be observed under the electron microscope (47, 48). These differences suggest that adipoQ may have structural and functional properties distinct from those of C1q.

In an *in vitro* transcription and translation system, the adipoQ cDNA generates a protein of approximately 30 kDa in size (Fig. 2*E*). This is in agreement with the molecular mass predicted from the cDNA sequence.

To test whether adipoQ is secreted, we constructed a flag-epitope tagged adipoQ and transiently transfected the DNA construct into NIH-3T3 cells. Western blot analysis (Fig. 2*E*) demonstrated that NIH-3T3 cells synthesized the adipoQ protein, and the protein is secreted into the medium.

Differentiation-dependent Expression of AdipoQ mRNA—We next examined the expression of adipoQ mRNA during adipocyte differentiation. As was shown in Fig. 1*B*, a single mRNA species of approximately 1.3 kilobases was expressed in both 3T3-F442A and 3T3-L1 preadipocytes but not in fibroblastic 3T3-C2 cells. The expression of adipoQ mRNA was found to increase approximately 20–50-fold during adipocyte differentiation in both 3T3-F442A and 3T3-L1 cells (Fig. 3, *A* and *B*). Compared with the expression of early adipose differentiation markers such as lipoprotein lipase (9) and PPAR-γ2 (26), the expression of adipoQ mRNA is a late event in adipogenesis, first appearing at approximately day 4 after induction of differentiation. This kinetics is similar to or slightly later than that of the aP2 mRNA and parallels the expression of adipsin mRNA (data not shown). It is also worth noting that the adipoQ mRNA is a very abundant message and can be readily detected in total RNA.

Adipose Tissue-specific Expression of AdipoQ in Mouse, Rat, and Human—To examine the tissue distribution of adipoQ mRNA, we performed Northern analysis using various tissue RNAs from both mouse and rat (Fig. 4, *A* and *B*). A single abundant mRNA species was present in mouse adipose tissue and very little adipoQ mRNA could be detected in other tissues (Fig. 4*A*). AdipoQ mRNA is at least 50–100-fold more abundant in adipose tissue than in any other tissues examined in mice. The distribution of adipoQ mRNA in rat is also highly restricted to adipose tissue (Fig. 4*B*). Interestingly, in rat three distinct adipoQ mRNAs of 2.5, 1.8, and 1.2 kilobases in size were detected, and all three mRNAs were adipose-specific. Whether these three distinct rat mRNA species encode the same or slightly different proteins remains to be determined. A single 4-kilobase adipoQ mRNA can also be detected in a human fat sample using mouse adipoQ cDNA as a probe (Fig. 4*C*). The expression of adipoQ mRNA in adipose tissue is highly restricted to mature fat cells in rat, and little or no expression was detected in the stromal-vascular fraction isolated from fat pads (Fig. 4*B*, *lanes 10* and *11*). This is consistent with the increased adipoQ expression observed during adipocyte differentiation in established cell lines.

Expression of AdipoQ in Lean and Obese Adipose Tissues from Mouse and Human—To investigate whether adipoQ gene expression is altered in obesity, we examined adipoQ mRNA levels in adipose tissue samples from lean (*ob*/+?) and obese (*ob*/*ob*) mice. Fat samples from obese and lean human individuals were also examined. As shown in Fig. 5*A*, a large (70–90%)

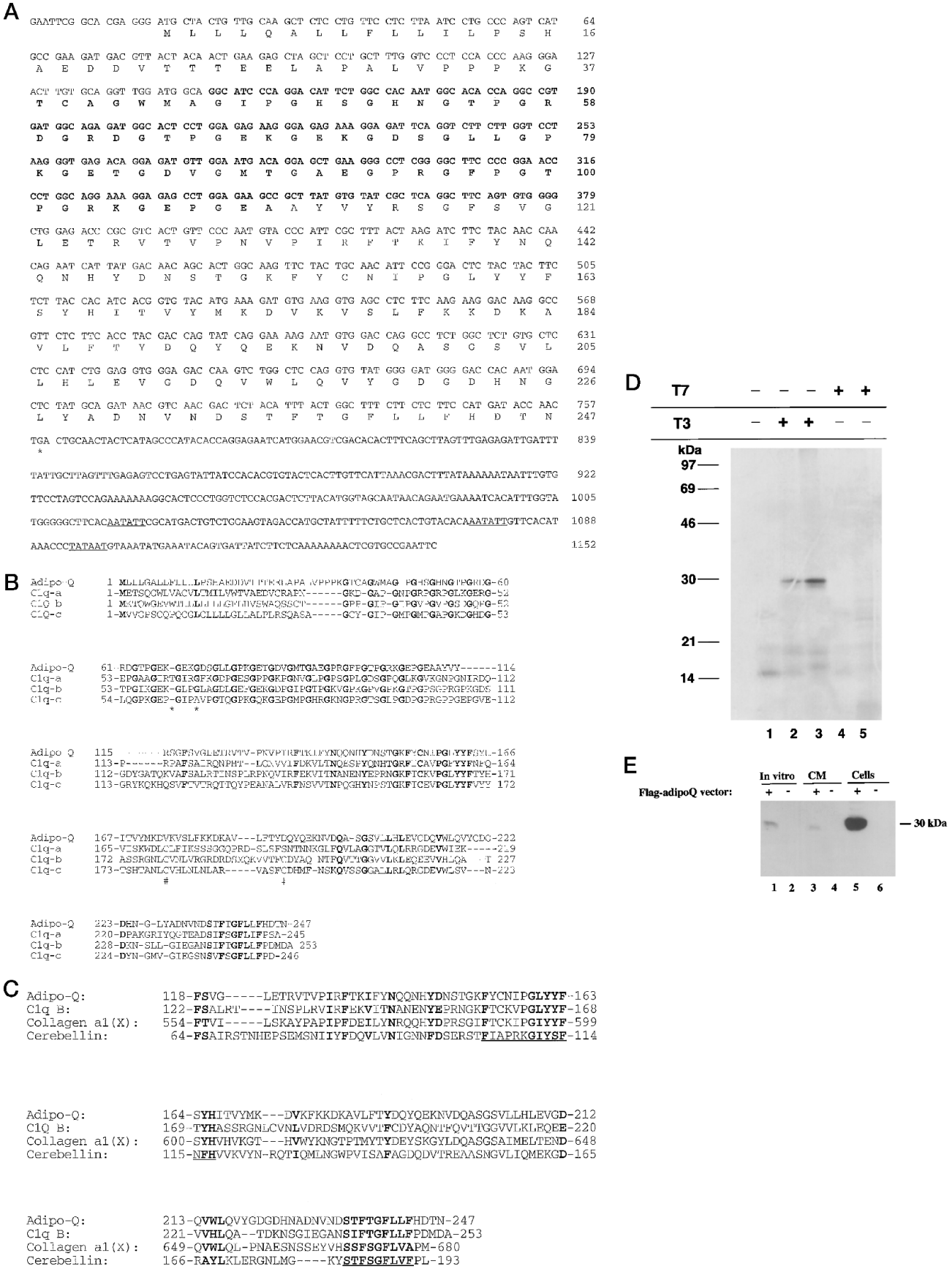

FIG. 2. **Nucleotide and deduced amino acid sequences of adipoQ: homology to components of C1q, collagen α1(X), and cerebellin.** *A*, nucleotides are numbered from the 5′ end of the sequence. The amino acid sequence is derived from the longest open reading frame. The *bold region* indicates the collagen-like domain. Putative polyadenylation signals are *underlined*. The accession number for adipoQ in GenBank is U49915. *B*, homology of adipoQ with murine C1q A, B, and C chains. The *bold region* indicates identical amino acids in all four sequences. Two interruptions in collagenous region of C1q-B and C1q-C chains are indicated by an *asterisk* (see text). Symbols # and ‡ mark the conserved cysteines

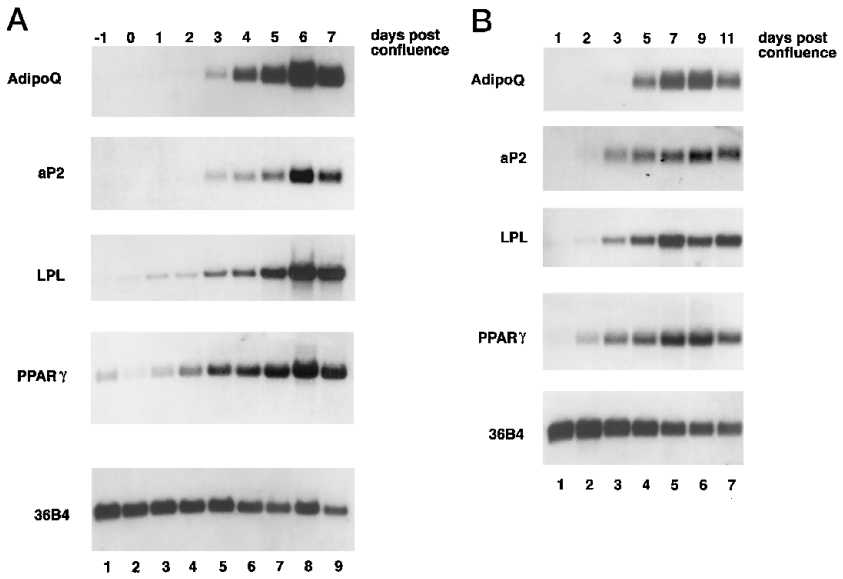

FIG. 3. **Expression of adipoQ mRNA during adipocyte differentiation.** Total RNA at different times during adipose differentiation was analyzed for both 3T3-F442A (*A*) and 3T3-L1 (*B*) cells. 10 μg of total RNA was loaded for each sample. Northern blots were sequentially hybridized to labeled cDNA probes corresponding to adipoQ, aP2, lipoprotein lipase, and PPAR-γ. RNA loading was normalized by hybridizing to a probe for ribosomal-associated protein (36B4 (68)).

reduction in adipoQ mRNA expression was observed in fat tissue from the obese mice. In contrast, the expression of another adipose-specific gene, aP2, was not affected by obesity (Fig. 5*A*). A more dramatic reduction (>50-fold) in adipsin mRNA expression was observed in the same mouse samples (Fig. 5*A*), in agreement with published results (49). We also examined adipoQ expression in fat samples from four obese (BMI = 39 $\pm$ 1.4) and three normal human individuals (BMI = 21 $\pm$ 0.3) (Fig. 5*B*). A reduction of 50–80% in adipoQ mRNA was observed in obese human fat tissue samples (Fig. 5*B*, *lanes 1–4*) as compared with the normal controls (Fig. 5*B*, *lanes 5–7*). Thus, expression of adipoQ mRNA is clearly dysregulated in obesity of both mouse and humans.

DISCUSSION

Adipose tissue was traditionally thought to be a relatively passive depot for lipid storage and mobilization and was viewed to be solely at the receiving end of hormonal and neuronal signals. Consistent with this, receptors for hormones such as insulin, adrenocorticotropic hormone, and epinephrine are abundantly expressed in adipose cells *in vivo* and *in vitro* (1). However, recent investigations suggest that fat tissue is much more actively involved in the energy balance systems by secreting molecules that signal to and perhaps regulate the functions of other tissues and organs (3, 15, 23). One clear example of this is the production of TNF-α by adipose tissue. This cytokine is produced by fat cells mainly in the context of animal and human obesity (21). It interferes with insulin action in both muscle and fat and plays a major role in systemic insulin resistance, at least in part through a reduction in the tyrosine kinase activity of the insulin receptor (50). Another example is the recently cloned obese (*ob*) gene product. The *ob* protein is synthesized mainly by adipocytes and is secreted into the circulation. Injection of this protein indicates that it influences (directly or indirectly) food intake and thermogenesis (15, 24, 51). Another secreted molecule from adipose tissue with signaling potential is adipsin. Originally identified as an adipocyte-specific serine protease (52), adipsin has been shown to encode a critical component of the alternative complement pathway (factor D) (53). Moreover, the proximal part of this complement pathway is shown to be activated in adipose tissue (54), generating small bioactive molecules such as the anaphylatoxin C3a that could affect systemic functions. Most recently, C3a has been shown to regulate triglyceride synthesis in fibroblasts and adipocytes (55). These data suggest that many important physiological functions may be controlled through secreted proteins from adipose tissue.

The adipoQ molecule identified in this study has several features that suggest that it could function as a signaling molecule from adipocytes. First, adipoQ contains a hydrophobic signal peptide sequence and is homologous to several secreted proteins such as C1q A, B, and C chains, collagen α1(X), and cerebellin. Consistent with this, adipoQ is secreted from fibroblasts after transfection of a expression vector. Second, the expression of adipoQ is highly regulated during differentiation and is restricted to adipose tissue *in vivo*. Finally, the expression of adipoQ is affected by obesity in rodents and humans, suggesting a dysregulation in this pathological state. These properties closely parallel those of other important signaling molecules secreted from adipose tissue including the *ob* gene product and TNF-α.

Given these unique sequence features and expression patterns, it is tempting to speculate on the possible functions of adipoQ. The sequence homology with C1q provide a possible clue. C1q is the first component of the classical complement activation pathway (46). It is composed of three homologous subunits: the A, B, and C chains. Each chain has a NH_2-

(see text) that are altered in adipoQ. *C*, comparisons of the globular region between C1q B chain, collagen α1(X), and cerebellin. Identical or conserved residues in all four sequences are indicated by *bold letters*. The two regions that are most homologous are *underlined*. Pair-wise comparisons between adipoQ and C1q-B chain, collagen α1(X), and cerebellin give 31, 38, and 25% identity, respectively. *D*, *in vitro* transcription and translation of adipoQ cDNA. TNT *in vitro* translation system (Promega) was used for translation (see "Experimental Procedures"). Varying amounts of purified cDNA plasmid (pBluescript) were added directly to the transcription-translation mixture with no RNA polymerase (*lane 1*), T3 RNA polymerase (0.5 μg DNA) (*lane 2*), T3 RNA polymerase (2 μg of DNA) (*lane 3*), T7 RNA polymerase (0.5 μg DNA) (*lane 4*) and T7 RNA polymerase (2 μg of DNA) (*lane 5*). [^{35}S]Methionine-labeled products were separated on 10% SDS gel and visualized after fluorography. Molecular mass is indicated by *kDa* at the *left side* of the gel. *E*, Western blot of proteins from conditional medium and cell lysates of transiently transfected NIH-3T3 cells (see "Experimental Procedures"). *Lane 1* and *2* were 1 μl of *in vitro* translated Flag-adipoQ and empty vector. *Lanes 3* and *4* were 50 μl (10 μg of protein) of culture medium from Flag-adipoQ transfected (*lane 3*) and empty vector (*lane 4*). *Lanes 5* and *6* were 10 μl (50 μg of protein) of cell lysates from Flag-adipoQ transfected (*lane 5*) and empty vector (*lane 6*). The proteins were separated on 12% SDS-polyacrylamide gel electrophoresis and immunoblotted with M2-Flag antibody from Kodak, Inc.

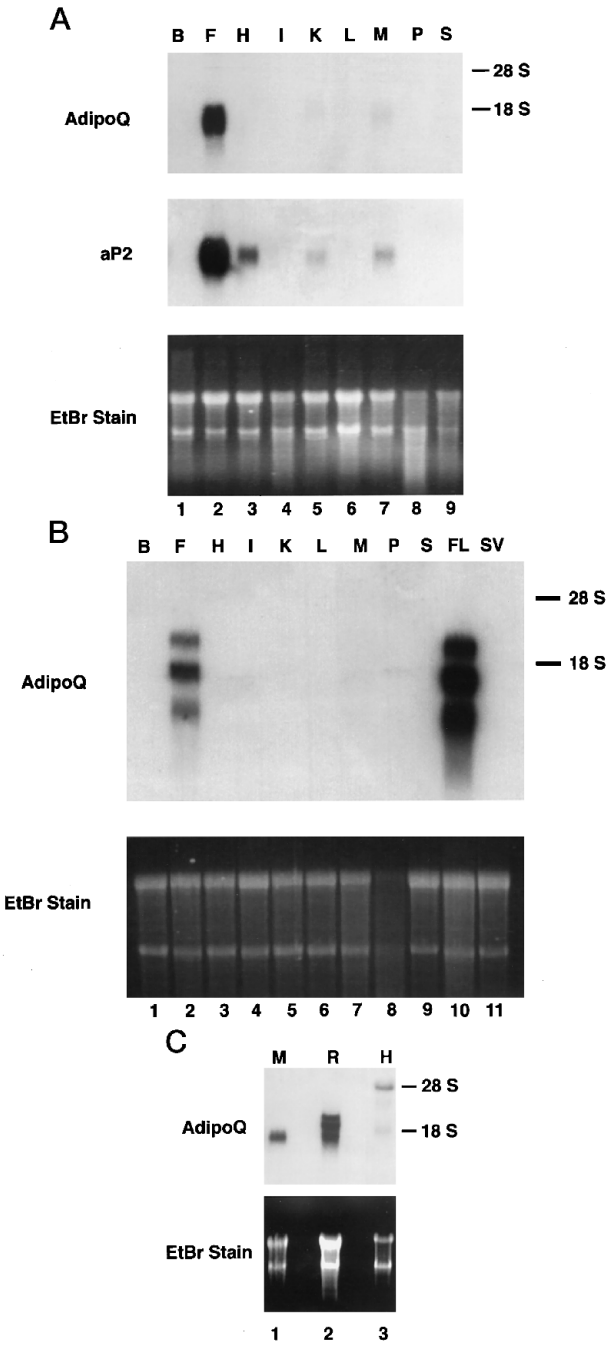

FIG. 4. **Expression of adipoQ mRNA in various tissues from mice, rats, and humans.** *A*, 10 μg of total RNA from different mouse tissues was analyzed by Northern blot. Tissues are designated as follows: *B*, brain; *Fa*, fat; *H*, heart; *I*, intestine; *K*, kidney; *L*, liver; *M*, muscle; *P*, pancreas; *S*, spleen. The blot was sequentially hybridized to the adipoQ and the aP2 cDNA probes. *B*, expression of adipoQ mRNA in rat tissues. 10 μg of total RNA from different rat tissues was analyzed by Northern blot. Tissue designation was identical to *A*, with two additional samples: mature fat cell from rat fat pads (floaters, *FL*), and the stromal-vascular fraction of rat fat pads (*SV*). *C*, comparison of adipoQ mRNA expression in mice, rats, and human fat. 10 μg of total fat tissue RNAs were analyzed by Northern blot. *M*, mouse; *R*, rat; *H*, human fat. Ethidium bromide (EtBr) stainings were used to normalize RNA loading.

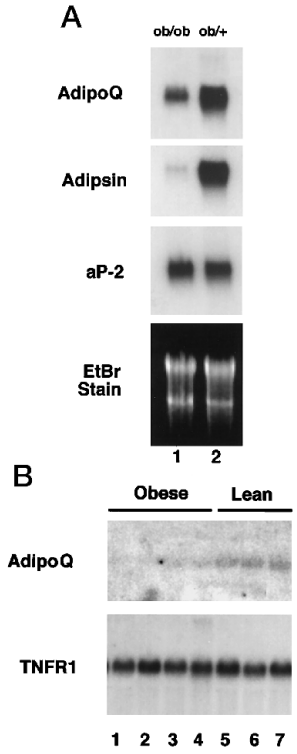

FIG. 5. **Expression of adipoQ mRNA in lean and obese fat samples from mice and humans.** *A*, 10 μg of total RNA from lean (*ob*/+) (*lane 2*) and obese (*ob*/*ob*) mice fat pads (*lane 1*) was analyzed by Northern blot. The same blot was hybridized with probes for adipoQ, adipsin, and aP2. *B*, 10 μg of total fat RNA from three lean human individuals (*lanes 5–7*) and four obese individuals (*lanes 1–4*) was analyzed for adipoQ expression. Expression of the TNF receptor type 1 (*TNFR1*) mRNA was not altered in these conditions and was used as a control for RNA loading (22).

terminal collagenous segment (Gly-X-Y repeats) of 78–84 amino acids and a globular carboxyl region of approximately 130 amino acids. A functional C1q molecule contains six subunits of each chain heteroligomerized along the collagenous helix. C1q interacts with the aggregated IgGs and initiates the complement cascade by proteolytically activating factors C2 and C4 (56). However, recent evidence suggests that C1q can also regulate other functions such as cell-mediated cytotoxicity (57), phagocytosis (58), chemotaxis (59), and interleukin-1 production (60) via a receptor-mediated mechanism. A putative receptor for C1q has been isolated and characterized in several human and murine cells including macrophages, lymphocytes, and fibroblasts (61). The collagenous region of C1q has been shown to be important for ligand-receptor interaction (62, 63). AdipoQ and C1q share significant similarities in the structure of this domain. Thus, it is possible that adipoQ could bind to the same or a similar receptor, thereby eliciting a biological response.

It is also possible that adipoQ may participate in the complement activation processes. Surrogate complement activation has been previously shown to be achieved by mannan-binding protein, a carbohydrate binding protein with a domain structure similar to that of C1q (64–66). However, because adipoQ lacks several key cysteines (see "Results") in the regions that are important for C1q function, it is not clear whether adipoQ functions in complement system. Further experiments will be needed to address this issue.

The identification of adipo-Q, a novel adipose tissue-specific protein, poses many questions regarding its molecular and biochemical properties that are yet to be examined. More importantly, its biological role in adipose tissue and in the overall energy balance systems remain to be defined. The production and purification of this novel protein should open a new avenue to studying adipose tissue physiology in normal and pathological states.

While this manuscript was under review, Scherer *et al.* (67) identified a adipocyte-specific protein named Acrp30 using a

random cDNA sequencing approach. AdipoQ is identical to Acrp30 in protein sequence.

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