Desnutrin, an Adipocyte Gene Encoding a Novel Patatin Domain-containing Protein, Is Induced by Fasting and Glucocorticoids

ECTOPIC EXPRESSION OF DESNUTRIN INCREASES TRIGLYCERIDE HYDROLYSIS*

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We have used rat cDNA microarrays to identify adipocyte-specific genes that could play an important role in adipocyte differentiation or function. Here, we report the cloning and identification of a 2.0-kb mRNA coding for a putative protein that we have designated as desnutrin. The novel gene is expressed predominantly in adipose tissue, and its expression is induced early during 3T3-L1 adipocyte differentiation. Desnutrin mRNA levels were regulated by the nutritional status of animals, being transiently induced during fasting. *In vitro* desnutrin gene expression was up-regulated by dexamethasone in a dose-dependent manner but not by cAMP, suggesting that glucocorticoids could mediate the increase in desnutrin mRNA levels observed during fasting. Desnutrin mRNA codes for a 486-amino acid putative protein containing a patatin-like domain, characteristic of many plant acyl hydrolases belonging to the patatin family. Confocal microscopy of enhanced green fluorescent protein-tagged desnutrin protein-transfected cells showed that the fusion protein localized in the cytoplasm. Moreover, cells overexpressing desnutrin by transfection showed an increase in triglyceride hydrolysis. Interestingly, we also found that the desnutrin gene expression level was lower in *ob/ob* and *db/db* obese mouse models. Overall, our data suggest that the newly identified desnutrin gene codes for an adipocyte protein that may function as a lipase and play a role in the adaptive response to a low energy state, such as fasting, by providing fatty acids to other tissues for oxidation. In addition, decreased expression of desnutrin in obesity models suggests its possible contribution to the pathophysiology of obesity.

Triglycerides serve as the most efficient form of energy storage in times of caloric excess in many organisms. During periods of energy demand, triglycerides can be rapidly mobilized by the hydrolytic action of lipases, releasing free fatty acids that are oxidized to meet the energy requirement of the organism. In mammals, adipose tissue serves as the major lipid storage site. Whereas white adipose tissue (WAT)[1] stores triglycerides that can be mobilized, producing fatty acids to be used by peripheral tissues, brown adipose tissue (BAT) itself uses the accumulated lipids to generate heat, a process known as adaptative thermogenesis (1). In addition, WAT plays an important role as an endocrine organ, secreting a wide variety of factors that are involved in various aspects of physiology, including appetite control, peripheral metabolism, immune response, and vascular function (2–4). The function of adipose tissue is dependent on the energy requirements and is tightly controlled by nutrient, neural, and hormonal signals. The crucial contribution of adipose tissue to the energy metabolism and function of an organism is manifested by pathological conditions in which dysregulation of adipose function leads to the development of severe diseases such as insulin resistance, diabetes mellitus, and cardiovascular disease (5).

The adipocyte is the main cell type present in adipose tissue. During adipose tissue development, precursor cells differentiate to generate adipocytes fully equipped with the enzymatic machinery and regulatory proteins that are needed to carry out their function in controlling fat metabolism and energy homeostasis. The adipogenic process entails dramatic morphological and biochemical changes and alteration in the expression of hundreds of genes (6–8). This includes increases in the expression of genes involved in the specialized role of adipose tissue in lipid metabolism, hormone responsiveness, the extracellular matrix, and secretion of endocrine and regulatory factors (2). Moreover, some of the genes are expressed only in adipose tissue and constitute a unique functional trait of this tissue. Identification of such genes or gene products is of crucial importance in unraveling the regulatory mechanisms of adipocyte differentiation and function. It is also a requirement for fully understanding the etiology of the pathologies associated with adipose tissue malfunction.

To identify novel genes that have a role in adipocyte differentiation or function, we have used several approaches based on differential gene expression. By differential screening and differential display techniques using the adipogenic 3T3-L1 cell line and primary preadipocytes, we previously cloned two regulatory proteins involved in the control of adipocyte differentiation, Pref-1 (9) and ENC-1 (10), respectively (10, 11). We recently employed the microarray technique to clone ADSF/resistin (12), an adipocyte-specific secreted factor that inhibits adipocyte differentiation *in vitro* and *in vivo* (12, 13) and that may be a contributing factor to insulin resistance (14).

Here, we report the cloning and identification of the full-length cDNA coding for a putative protein that we named desnutrin. This novel gene is induced early during 3T3-L1

[1] The abbreviations used are: WAT, white adipose tissue; BAT, brown adipose tissue; RACE, rapid amplification of cDNA ends; EST, expressed sequence tag; contig, group of overlapping clones; HA, hemagglutinin; EGFP, enhanced green fluorescent protein; PBS, phosphate-buffered saline; DMEM, Dulbecco's modified Eagle's medium; MIX, methylisobutylxanthine; C/EBP, CAAT/enhancer-binding protein.

adipocyte differentiation and is expressed predominantly in adipose tissue. Desnutrin mRNA levels are under the control of the nutritional status of animals, being transiently induced during fasting. *In vitro*, desnutrin gene expression was regulated by dexamethasone, but not by cAMP, suggesting that glucocorticoids could mediate the increase in desnutrin mRNA during fasting. Desnutrin mRNA codes for a 486-amino acid protein containing a patatin-like domain, characteristic of many plant acyl hydrolases that belong to the patatin family. We found that ectopic overexpression of desnutrin increased triglyceride hydrolysis in the cell, suggesting the function of desnutrin as a lipase and its role in the adaptive response of adipose tissue to the low energy state of fasting, releasing substrates for oxidation to meet the energy requirement for other tissues.

EXPERIMENTAL PROCEDURES

Genefilter Microarray Analysis—Identification of genes expressed exclusively in adipose tissue was achieved by comparing the gene expression patterns of different mouse tissues using rat Genefilter membranes (Research Genetics) as described previously (12). Briefly, filters were hybridized with ^{33}P-labeled cDNAs synthesized by reverse transcription using 5 μg of total RNA from WAT, brain, muscle, and liver. Only those spots found exclusively in filters hybridized with WAT cDNAs were further analyzed.

Cloning of Desnutrin cDNA and 3′-Rapid Amplification of cDNA Ends (RACE)—A BLASTn search (15) conducted using the identified rat expressed sequence tag (EST) clone sequence (GenBank™/EBI accession number AI059513) as a query in the mouse genome data base of NCBI revealed a single match located on the mouse chromosome 7 contig. The gene was identified as the 0610039C21Riken gene, and from now on, we will refer to it as desnutrin. The corresponding annotated mRNA sequence of the 0610039C21Riken gene (accession number NM_025802) was used to design PCR primers for cloning the cDNA fragment containing the entire open reading frame. Briefly, 5 μg of total RNA from WAT was reverse-transcribed with Superscript II reverse transcriptase (Invitrogen) and oligo(dT) in a total volume of 20 μl. Two μl of the reaction was used as the template, and desnutrin partial cDNA was amplified by PCR using primers 5′-GACAGCGTCTCCGCCTCCG-C-3′ (forward) and 5′-GACAGGATCTTGTTCCACCCC-3′ (reverse). The 1634-bp PCR product was cloned into the pGEM-T-Easy vector (Promega), and the insert fragment was fully sequenced.

We performed 3′-RACE to obtain the 3′-end of desnutrin cDNA using the desnutrin-specific primer 5′-TGTCCTTCACCATCCGCTTGTTG-3′, oligo(dT), and WAT cDNA as the template. PCR was carried out for 35 cycles of denaturation (at 95 °C for 45 s), annealing (at 42 °C for 45 s), and extension (at 72 °C for 1 min). The resulting fragment was cloned into the pGEM-T-Easy vector and sequenced.

Construction of Plasmids—To obtain a hemagglutinin (HA)-tagged desnutrin expression vector, PCR was carried out using the cDNA containing the entire coding region (described above) as the template and primers 5′-CGCGGAGACCCCAAGGTATC-3′ (forward) and 5′-TTACAA-GCTGTAATCTGGAACATCGTATGGGTAGCAAGGCGGGAGGCCAGG-TGGATCCTGTGGGGT-3′ (reverse). The PCR fragment was cloned into the pGEM-T-Easy vector and then subcloned into the NotI site of the pcDNA3.1 vector (Invitrogen) for mammalian expression.

A fusion protein construct bearing the coding region of desnutrin was prepared by subcloning a reverse transcription-PCR product of desnutrin into the pEGFP-N1 vector (BD Biosciences) in-frame with enhanced green fluorescent protein (EGFP). Briefly, PCR was performed using 3T3-L1 adipocyte cDNA as the template and primers 5′-TCCGGACTCAGATCT-ATGTTCCCGAGGGAGACCAAGTGG-3′ (forward) and 5′-CGTACCGTC-GACTGCAAGCTGTAATCTGGAACATC-3′ (reverse). The PCR product was purified on agarose gel, digested with BglII and SalI, and cloned into the BglII/SalI sites of the pEGFP-N1 vector.

Animals—For tissue distribution of desnutrin mRNA and its regulation by fasting and refeeding, male C57BL/6 mice were used. In the fasting/refeeding experiments, mice were fasted for a period of up to 48 h, after which they were killed, and tissues were extracted for RNA analysis. Mice were refed a high carbohydrate diet for 12 h after 48 h of fasting before being killed. Desnutrin mRNA levels was also determined by reverse transcription-PCR and Northern blot analysis in gonadal adipose tissue obtained from *ob/ob* and *db/db* male mice (Jackson Laboratory, Bar Harbor, ME) fasted for 12 h.

Separation of the Stromal Vascular Fraction and Adipocytes of WAT—Inguinal WAT from mice was isolated, washed with phosphate-buffered saline (PBS), and minced prior to digestion with 2 mg/ml collagenase and 2% bovine serum albumin in Dulbecco's modified Eagle's medium (DMEM). Digestion was carried out for 20–30 min at 37 °C under constant agitation until tissue integrity was disrupted. The cell suspension was filtered through a 70-μm mesh filter to remove undigested tissue fragments, and mature adipocytes were separated from the stromal vascular fraction by flotation. The stromal vascular fraction was collected with a syringe, and cells were pelleted by centrifugation at 500 × g for 10 min. Cells were immediately processed for RNA extraction.

Cell Culture and Transfection—3T3-L1 cells (American Type Culture Collection) were cultured in DMEM containing 10% calf serum. To induce differentiation of 3T3-L1 cells into adipocytes, 2-day post-confluent preadipocytes (day 0) were treated with 1 μM dexamethasone (DEX) and 0.5 μM methylisobutylxanthine (MIX) for 48 h. After the induction period, cells were switched to differentiation medium (DMEM supplemented with 10% fetal calf serum) and maintained for 5–7 days, at which point 90% of the cells exhibited the typical adipocyte morphology. For the experiments examining the effect of glucocorticoids on desnutrin expression, confluent preadipocytes were treated with dexamethasone, MIX, dibutyryl cAMP, or dexamethasone/MIX at the concentrations indicated.

COS-7 cells (American Type Culture Collection) were cultured in DMEM containing 10% fetal calf serum. Transient transfection of COS-7 cells with desnutrin-EGFP or HA-desnutrin expression vectors was carried out by the DEAE-dextran/chloroquine method as described previously (16).

RNA Isolation and Northern Blot Analysis—Total RNA from mouse tissues or cells was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. For Northern blot analysis, 5–15 μg of total RNA was subjected to electrophoresis on formaldehyde-containing 1.2% agarose gel and transferred onto Hybond N$^+$ nylon membranes (Amersham Biosciences). Blot hybridization was carried out in ExpressHyb solution (Clontech) using ^{32}P-labeled cDNA-specific probes for desnutrin, adiponutrin, fatty-acid synthase, adipocyte fatty acid-binding protein (aFABP/aP2), CAAT/enhancer-binding protein-α (C/EBPα), and peroxisome proliferator-activated receptor-γ.

Confocal Microscopy—COS-7 cells were transiently transfected with desnutrin-EGFP (0.7 μg of DNA) as described above and grown on glass coverslips. Thirty-six h post-transfection, cells were fixed with 4% paraformaldehyde for 10 min. The samples were briefly equilibrated with PBS, and nuclei were counterstained with 0.3 μM 4′,6-diamidino-2-phenylindole (Molecular Probes) for 3–4 min. The samples were rinsed several times with PBS and mounted on glass microscope slides using Antifade and Prolong mounting media (Molecular Probes) according to the manufacturer's instructions. As a positive control, the empty pEGFP-N1 vector expressing only EGFP was used. Images were captured using a Zeiss 510 UV-visible laser scanning confocal microscope.

Subcellular Fractionation by Differential Centrifugation and Western Blot Analysis—COS-7 cells transfected with an expression vector for HA-tagged desnutrin were washed three times with PBS and twice with 0.25 M sucrose, 10 mM triethanolamine, and 10 mM acetic acid (pH 7.8). Cells were harvested in ice-cold 0.25 M sucrose, 10 mM triethanolamine, 10 mM acetic acid, and 1 mM EDTA (pH 7.8) and homogenized using a Dounce homogenizer. The crude nuclear fraction was obtained by centrifugation at 1000 × g for 10 min. The supernatant was centrifuged at 18,000 × g for 10 min to obtain the mitochondrial fraction, and the resulting supernatant was then centrifuged at 100,000 × g for 1 h to separate the microsomal fraction from the cytosolic fraction. Equal amounts of protein from each fraction were subjected to 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore Corp.) for immunodetection using anti-HA antibody (Covance).

Separation of Lipids from Transfected COS-7 Cells—COS-7 cells were transfected with the HA-desnutrin expression vector or the control pcDNA3.1 vector as described above. Thirty-six h post-transfection, cells were incubated for 4 h in serum-free medium (DMEM) containing fatty acid-free bovine serum albumin (2 mg/ml) and [U-^{14}C]palmitic acid (final volume of 0.1 μCi/ml; Amersham Biosciences). Cells were washed with PBS and harvested for lipid extraction (time 0) or further incubated with serum-containing medium for an additional 4, 8, and 16 h. Lipids from cells and media were extracted by the method of Bligh and Dyer (17) and separated by TLC using solvent system A (hexane/diethyl ether/acetic acid (80:20:2)) to resolve neutral lipids or solvent system B (chloroform/methanol/ammonium hydroxide/water (65:35:5:1)) to separate polar lipids. Radioactive lipids were detected by autoradiography. To measure the radiolabeled lipids released to the media by

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cells, 1 ml of cell culture medium was collected at the end of each time point and mixed with 20 ml of CytoScint (Fisher), and the radioactivity was measured by scintillation counting.

<div align="center">RESULTS</div>

Cloning of Full-length Mouse Desnutrin cDNA—We performed microarray analysis using EST cDNA filter microarrays to identify those genes expressed exclusively in adipose tissue. We compared the gene expression patterns in brain, muscle, liver, and adipose tissue using rat EST cDNA arrays and identified a set of genes that showed signal only on the arrays that were hybridized with WAT cDNAs. Candidate EST clones were sequenced, and adipose tissue-specific expression was verified by Northern blot analysis using RNA from liver, brain, muscle, and WAT (data not shown). One of these clones showed exclusive expression in WAT. Using the 434-bp sequence of the selected EST clone (GenBank™/EBI accession number AI059513) as a query, the BLASTn search of the mouse genome data base of NCBI identified a single match on the 3′-region of the 0610039C21Riken gene, located in chromosome 7. We used the sequence of the annotated 0610039C21Riken gene mRNA (accession number NM_025802) for the design of a set of primers to amplify the coding region of the gene using mouse cDNA reverse-transcribed with RNA prepared from WAT. In addition, the 3′-end was further characterized by carrying out 3′-RACE. A total of nine amplification products were sequenced, and the resulting consensus 3′-end sequence was used for PCR to generate the full-length desnutrin cDNA of 1965 bp (Fig. 1). Desnutrin encodes a protein with a length of 486 amino acids and an estimated molecular mass of 53.6 kDa. The cDNA contains a short 5′-untranslated region of 80 bases and a long 3′-untranslated region of 426 bases with a single polyadenylation signal (Fig. 1).

The homology search revealed the presence of related proteins in a wide range of organisms, including animals and plants. In humans, two proteins with a high degree of homology (88%) to desnutrin have been identified. These proteins, named TTS2.2 (GenBank™/EBI accession number CAC01132) and TTS2.1 (accession number CAC01131), appear to be distinct proteins and have been implicated in the vesicular transport and secretion of the ICAM-3 (intercellular adhesion molecule-3) cell-surface receptor. In addition, two other putative human proteins sharing high homology with desnutrin have been identified: GS2-like protein (accession number NP_620169) and the hypothetical chromosome 22 open reading frame (accession number NP_079501). In mouse, two homologous proteins can be found: a hypothetical protein (accession number XP_128189) that appears to be the mouse homolog of the human GS2-like protein and adiponutrin. Adiponutrin has been recently cloned (18) as an adipose tissue-specific protein whose expression is up-regulated during adipocyte differentiation, but down-regulated during fasting in mice. Two homologous proteins are found in *Drosophila melanogaster* and four in *Caenorhabditis elegans*. Interestingly, additional putative proteins sharing some degree of homology are present in *Arabidopsis thaliana*, such as the protein At1g33270 (accession number NP_977474), and in prokaryotes, including *Bacteroides thetaiotaomicron* (accession number NP_809687) and *Bacillus subtilis* (accession number NP_389387). The presence of desnutrin homologs and other related proteins in such a variety of organisms suggests that desnutrin belongs to a family of proteins whose members are widely expressed in all organisms, and this points toward a basic function of these proteins that is not exclusive of higher organisms.

Desnutrin mRNA Is Expressed Predominantly in Adipose Tissue—Northern blot analysis was performed to determine the expression pattern of desnutrin in various types of mouse tissues. Hybridization of mouse RNAs with a desnutrin probe spanning nucleotides 20–1632 revealed a single mRNA of ∼2.0 kb in size (Fig. 2A). The appearance of a single band is in agreement with the presence of a single polyadenylation signal in the desnutrin cDNA sequence. Considering the average poly(A) length, we conclude that our desnutrin cDNA sequence represents full-length cDNA. As shown in Fig. 2A, desnutrin mRNA was highly expressed in various depots of WAT as well as BAT. Low but detectable desnutrin levels were also found in other tissues such as heart and testis, whereas desnutrin mRNA was found at a very low, barely detectable level in liver, spleen, thymus, kidney, brain, skeletal muscle, and lung. Therefore, although ubiquitously expressed, desnutrin is highly expressed in adipose tissues only. It is interesting to note that the desnutrin expression levels differed depending on the type or location of adipose tissue. Overall, BAT showed higher desnutrin mRNA levels than WAT. Among the white adipose depots, gonadal fat showed the highest level of expression compared with inguinal and renal WAT. Adiponutrin, a closely related gene known to be expressed exclusively in adipose tissue, showed a pattern of expression similar to that of desnutrin in the different adipose depots, with a higher level of expression in BAT and gonadal WAT (Fig. 2A). We next examined desnutrin gene expression in gonadal fat from the genetically obese mouse models *ob/ob* and *db/db* by Northern blot analysis (Fig. 2B) as well as by reverse transcription-PCR (data not shown). We observed ∼50% lower desnutrin mRNA levels in *db/db* mice and 80% lower levels in *ob/ob* mice compared with wild-type C57BL/6 mice, suggesting a possible significance of desnutrin function in obesity.

To identify the cell type that is responsible for the high expression level of desnutrin mRNA in adipose tissue, cellular components of inguinal WAT were fractionated into adipocytes and a stromal vascular fraction containing preadipocytes as well as endothelial cells and resident macrophages. As shown in Fig. 2C (*left panels*), the desnutrin transcript was not detected in the stromal vascular fraction, but was found exclusively in the adipocyte fraction, along with adipocyte markers such as fatty-acid synthase, adipocyte fatty acid-binding protein/aP2, C/EBPα, and adiponutrin. The adipocyte-specific expression of desnutrin mRNA was corroborated in 3T3-L1 cells, an established cell line that can be induced to differentiate into adipocytes upon appropriate hormone treatment and used as a model system for adipogenesis. Desnutrin mRNA was found only in mature adipocytes, but not in proliferating or confluent 3T3-L1 preadipocytes (Fig. 2C, *right panels*). We next examined desnutrin gene expression during the course of conversion of 3T3-L1 preadipocytes to adipocytes. As predicted and shown in Fig. 3, desnutrin mRNA was not detected in preadipocytes (day 0). However, its expression was rapidly increased when cells were induced to differentiate. The maximal level of desnutrin mRNA was reached after 6 days, when cells were fully differentiated into adipocytes, as judged by accumulation of lipids and expression of adipocyte markers such adipocyte fatty acid-binding protein/aP2, fatty-acid synthase, peroxisome proliferator-activated receptor-γ, C/EBPα, and adiponutrin. Interestingly, a detectable level of desnutrin mRNA could be observed in 3T3-L1 cells as early as 24 h after induction of differentiation, preceding the expression of the various late adipocyte markers. Thus, desnutrin is an adipocyte gene induced early during 3T3-L1 adipocyte differentiation.

Induction of Desnutrin mRNA by Fasting and by Glucocorticoids—Expression of the adiponutrin gene, which is closely related to desnutrin, is dramatically down-regulated by fasting, and its levels are restored upon refeeding (18). Because of the homology between the two putative proteins, we decided to

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        gacagcgtctccgcctccgcggcggagaccccaaggtatcgagactgcgggacccactgcccgcaggacatcgagtcacg    -1

ATG TTC CCG AGG GAG ACC AAG TGG AAC ATC TCA TTC GCT GGC TGC GGC TTC CTC GGG GTC TAC CAC ATT    69
 M   F   P   R   E   T   K   W   N   I   S   F   A   G   C   G   F   L   G   V   Y   H   I      23

GGC GTG GCC TCC TGC CTC CAT GAG CAC GCG CCC TTC CTG GTG GCC AAC GCC ACT CAC ATC TAC GGG GCC    138
 G   V   A   S   C   L   H   E   H   A   P   F   L   V   A   N   A   T   H   I   Y   G   A      46

TCG GCA GGG GCG CTC ACC GCC ACA GCG CTG GTC ACT GGG GCC TGC CTG GGT GAA GCA GGT GCC AAC ATT    207
 S   A   G   A   L   T   A   T   A   L   V   T   G   A   C   L   G   E   A   G   A   N   I      69

ATT GAG GTG TCC AAG GAG GCC CGG AAG CGG TTC CTG GGT CCT CTG CAT CCC TCC TTC AAC CTG GTG AAG    276
 I   E   V   S   K   E   A   R   K   R   F   L   G   P   L   H   P   S   F   N   L   V   K      92

ACC ATC CGT GGC TGT CTA CTA AAG ACC CTG CCT GCT GAT TGC CAT GAG CGC GCC AAT GGA CGC CTG GGC    345
 T   I   R   G   C   L   L   K   T   L   P   A   D   C   H   E   R   A   N   G   R   L   G     115

ATC TCC CTG ACT CGT GTT TCA GAC GGA GAG AAC GTC ATC ATA TCC CAC TTT AGC TCC AAG GAT GAG CTC    414
 I   S   L   T   R   V   S   D   G   E   N   V   I   I   S   H   F   S   S   K   D   E   L     138

ATC CAG GCC AAT GTC TGC AGC ACA TTT ATC CCG GTG TAC TGT GGC CTC ATT CCT CCT ACC CTC CAA GGG    483
 I   Q   A   N   V   C   S   T   F   I   P   V   Y   C   G   L   I   P   P   T   L   Q   G     161

GTG CGC TAT GTG GAT GGC GGC ATT TCA GAC AAC TTG CCA CTT TAT GAG CTG AAG AAT ACC ATC ACA GTG    552
 V   R   Y   V   D   G   G   I   S   D   N   L   P   L   Y   E   L   K   N   T   I   T   V     184

TCC CCA TTC TCA GGC GAG AGT GAC ATC TGC CCT CAG GAC AGC TCC ACC AAC ATC CAC GAG CTT CGC GTC    621
 S   P   F   S   G   E   S   D   I   C   P   Q   D   S   S   T   N   I   H   E   L   R   V     207

ACC AAC ACC AGC ATC CAG TTC AAC CTT CGC AAT CTC TAC CGC CTC TCG AAG GCT CTC TTC CCG CCA GAG    690
 T   N   T   S   I   Q   F   N   L   R   N   L   Y   R   L   S   K   A   L   F   P   P   E     230

CCC ATG GTC CTC CGA GAG ATG TGC AAA CAG GGC TAC AGA GAT GGA CTT CGA TTC CTT AGG AGG AAT GGC    759
 P   M   V   L   R   E   M   C   K   Q   G   Y   R   D   G   L   R   F   L   R   R   N   G     253

CTA CTG AAC CAA CCC AAC CCT TTG CTG GCA CTG CCC CCA GTT GTC CCC CAG GAA GAG GAT GCA GAG GAA    828
 L   L   N   Q   P   N   P   L   L   A   L   P   P   V   V   P   Q   E   E   D   A   E   E     276

GCT GCT GTG GTG GAG GAG AGG GCT GGA GAG GAG GAT CAA TTG CAG CCT TAT AGA AAA GAT CGA ATT CTA    897
 A   A   V   V   E   E   R   A   G   E   E   D   Q   L   Q   P   Y   R   K   D   R   I   L     299

GAG CAC CTG CCT GCC CGA CTC AAT GAG GCC CTG CTG GAG GCC TGT GTG GAA CCA AAG GAC CTG ATG ACC    966
 E   H   L   P   A   R   L   N   E   A   L   L   E   A   C   V   E   P   K   D   L   M   T     322

ACC CTT TCC AAC ATG CTA CCA GTG CGC CTG GCA ACG GCC ATG ATG GTG CCC TAT ACT CTG CCG CTG GAG   1035
 T   L   S   N   M   L   P   V   R   L   A   T   A   M   M   V   P   Y   T   L   P   L   E     345

AGT GCA GTG TCC TTC ACC ATC CGC TTG TTG GAG TGG CTG CCT GAT GTC CCT GAA GAT ATC CGG TGG ATG   1104
 S   A   V   S   F   T   I   R   L   L   E   W   L   P   D   V   P   E   D   I   R   W   M     368

AAA GAG CAG ACG GGT AGC ATC TGC CAG TAT CTG GTG ATG AGG GCC AAG AGG AAA TTG GGT GAC CAT CTG   1173
 K   E   Q   T   G   S   I   C   Q   Y   L   V   M   R   A   K   R   K   L   G   D   H   L     391

CCT TCC AGA CTG TCT GAG CAG GTG GAA CTG CGA CGT GCC CAG TCT CTG CCC TCT GTG CCA CTG TCT TGC   1242
 P   S   R   L   S   E   Q   V   E   L   R   R   A   Q   S   L   P   S   V   P   L   S   C     414

GCC ACC TAC AGT GAG GCC CTA CCC AAC TGG GTA CGA AAC AAC CTC TCA CTG GGG GAC GCG CTG GCC AAG   1311
 A   T   Y   S   E   A   L   P   N   W   V   R   N   N   L   S   L   G   D   A   L   A   K     437

TGG GAA GAA TGC CAG CGT CAG CTA CTG CTG GGT CTC TTC TGC ACC AAT GTG GCC TTC CCG CCG GAT GCC   1380
 W   E   E   C   Q   R   Q   L   L   L   G   L   F   C   T   N   V   A   F   P   P   D   A     460

TTG CGC ATG CGC GCA CCT GCC AGC CCC ACT GCC GCA GAT CCT GCC ACC CCA CAG GAT CCA CCT GGC CTC   1449
 L   R   M   R   A   P   A   S   P   T   A   A   D   P   A   T   P   Q   D   P   P   G   L     483

CCG CCT TGC TGA G aatcaccattcccacatcgcccggctaccagccaagctccaagttgtcctgccccactaagaggagccccgggg   1536
 P   P   C   *                                                                                487

tggaacaagatcctgtctgcccccggctctcccccttacatgctgtggaatgaggacataggaccctgcacagctgcaagtgggctttcgatg    1628

tgaaacctttcaccagccactcactatgctactcctggtggggagggatggggagtcgccctcccccggagcccacagagccctcccccgtc    1720

acgtcacctgtgccttactcctgcccaccacctttttcagtgcagggtcagtcttaagaactccacatctgctgctgctccctggtgtccaag    1812

tttccttgcagagtgtgtgaagaattatttattttttgccaaagcagatct**aataaa**agccacagctcagctta aaaaaaaaaaaaaaaaaaaa    1904

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FIG. 1. **Nucleotide and deduced amino acid sequences of the full-length mouse desnutrin cDNA.** The full-length cDNA encoding desnutrin was cloned from WAT. The predicted amino acid sequence corresponds to the longest open reading frame (486 amino acids) and is shown under the nucleotide sequence. The *boldface* nucleotides indicate the polyadenylation signal, and the *asterisk* shows the stop codon. Amino acids 8–180 (in *boldface*) indicate the conserved patatin domain.
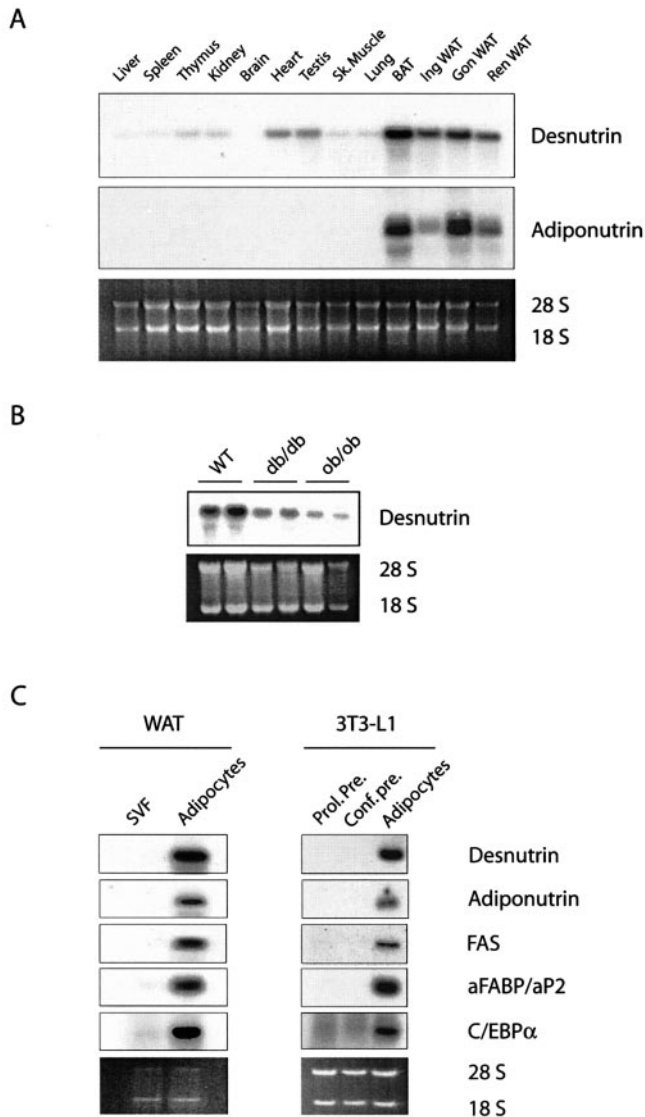
A

B

C

FIG. 2. **Desnutrin mRNA levels in various adult mouse tissues and cells.** *A*, 10 μg of total RNA from various mouse tissues was analyzed by Northern blotting and hybridized with radiolabeled desnutrin and adiponutrin cDNA probes. *Sk*, skeletal; *Ing*, inguinal; *Gon*, gonadal; *Ren*, renal. *B*, shown is desnutrin mRNA expression in gonadal WAT from 12-h fasted wild-type (*WT*), *db/db*, and *ob/ob* mice. *C*, 5 μg of total RNA from cells of the stromal vascular fraction (*SVF*) or adipocytes isolated from mouse inguinal adipose tissue (*left panels*) or 10 μg of total RNA from 3T3-L1 cells at the indicated days of differentiation (*right panels*) was examined by Northern blot analysis for the expression of desnutrin and various adipocyte markers. *Prol. Pre.*, proliferating preadipocytes; *Conf. pre.*, confluent preadipocytes; *FAS*, fatty-acid synthase; *aFABP*, adipocyte fatty acid-binding protein.

investigate whether desnutrin gene expression could also be regulated by the nutritional status of the organism. WAT from animals subjected to fasting/refeeding was analyzed for desnutrin expression. As shown in Fig. 4*A*, the levels of desnutrin mRNA were higher in 24-h fasted mice than in mice that underwent food deprivation for the same time period and then were refed for 12 h. These results show evidence of nutritional regulation of desnutrin gene expression. To determine whether desnutrin expression is down-regulated during refeeding or up-regulated by food deprivation, a set of time course experiments were conducted in which mice were fasted for 12, 24, and 48 h or fed for 12 additional h after 48 h of fasting. As shown in Fig. 4*B*, compared with fed mice, desnutrin mRNA levels increased rapidly after 12 h of fasting, reaching a maximal level
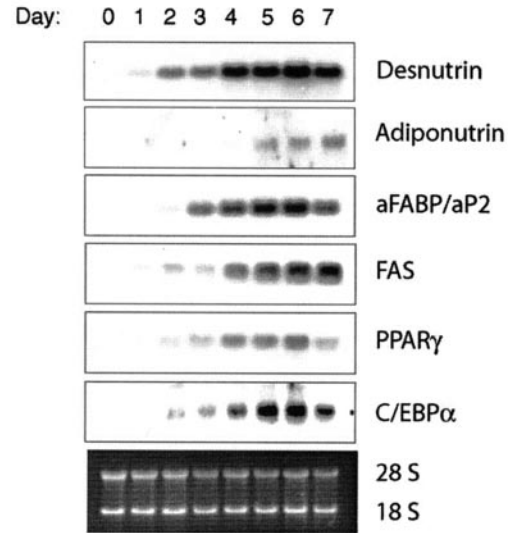

FIG. 3. **Desnutrin mRNA levels during adipocyte differentiation of 3T3-L1 cells.** Two-day post-confluent 3T3-L1 preadipocytes (day 0) were induced to differentiate by treatment with 1 μM dexamethasone and 0.5 mM MIX for 2 days and then maintained in differentiation medium for an additional 5 days. Ten μg of total RNA prepared from cells collected at the indicated time points was examined for the expression of desnutrin and other adipocyte markers by Northern blot analysis. *aFABP*, adipocyte fatty acid-binding protein; *FAS*, fatty-acid synthase; *PPARγ*, peroxisome proliferator-activated receptor-γ.
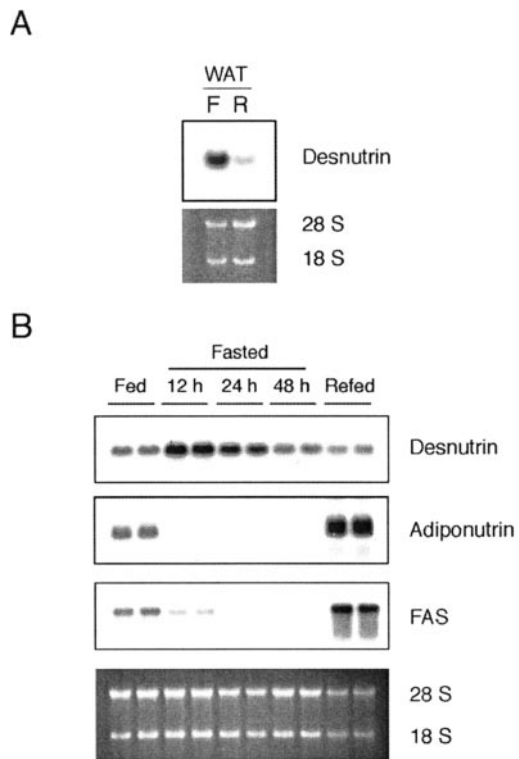
A

B

FIG. 4. **Nutritional regulation of desnutrin mRNA levels.** *A*, expression of desnutrin mRNA in WAT from mice fasted for 24 h (*F*) or fasted and then refed for 12 h (*R*) as assessed by Northern blot analysis; *B*, time course analysis of desnutrin and adiponutrin mRNA expression levels in WAT during fasting. Mice were fasted for 12, 24, or 48 h, and total RNA was extracted for examination of desnutrin mRNA levels by Northern blot analysis. Desnutrin mRNA levels in WAT were compared with those in mice that were fasted for 48 h and subsequently fed for 12 h. *FAS*, fatty-acid synthase.

and then gradually decreasing to basal levels 48 h after fasting was initiated. Interestingly the expression pattern of the desnutrin gene was inversely correlated with that of the adiponu-
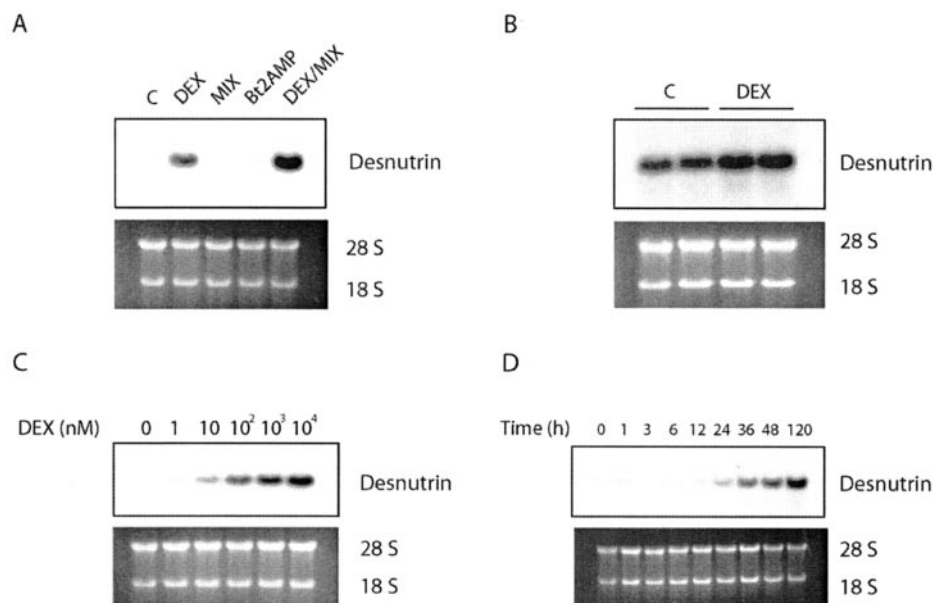
FIG. 5. **Regulation of desnutrin mRNA levels by glucocorticoids.** *A*, confluent preadipocytes treated for 48 h with 1 μM dexamethasone (*DEX*), 0.5 mM MIX, 0.5 mM dibutyryl cAMP (*Bt2AMP*), or 1 μM dexamethasone and 0.5 mM MIX for 48 h (*DEX/MIX*). After the incubation period, cells were harvested, and desnutrin mRNA levels were analyzed by Northern blotting. *B*, desnutrin mRNA levels in fully differentiated adipocytes untreated (*C*) or treated for 48 h with 1 μM dexamethasone. *C*, dose-dependent induction of desnutrin mRNA by dexamethasone in 3T3-L1 preadipocytes. *D*, time course analysis of desnutrin mRNA expression during dexamethasone treatment in 3T3-L1 preadipocytes. Cells were treated with 1 μM dexamethasone for the indicated times.

trin gene, whose expression practically disappeared when mice were fasted, but was induced upon refeeding. The fatty-acid synthase gene, known to be tightly regulated by fasting and refeeding (19), was used as a control. As predicted, fatty-acid synthase mRNA levels were not detectable during fasting, but increased drastically during the refeeding period. Together, these results indicate that desnutrin gene expression is rapidly and transiently induced in WAT by fasting.

During fasting, circulating glucagon and glucocorticoids levels are elevated, and these hormones serve as mediators of the adaptive response to starvation by inducing changes in gene expression to face the new metabolic conditions. Because desnutrin mRNA is induced during fasting, we investigated whether cAMP, the mediator of glucagon action, or glucocorticoids can regulate desnutrin expression. Confluent 3T3-L1 preadipocytes, which do not have detectable levels of desnutrin mRNA, were treated for 48 h with dexamethasone, MIX, dibutyryl cAMP, or dexamethasone/MIX. As shown in Fig. 5*A*, neither MIX (an inhibitor of phosphodiesterase) nor the cAMP analog dibutyryl cAMP induced expression of the desnutrin gene, indicating that cAMP and glucagon are not involved in the induction of desnutrin mRNA during fasting. However, dexamethasone, a synthetic glucocorticoid, was able to significantly increase desnutrin mRNA levels in preadipocytes. The combination of dexamethasone and MIX seemed to have a more exacerbated effect on desnutrin gene expression, probably because of the initiation of adipocyte differentiation by dexamethasone/MIX treatment. Overall, these results indicate that glucocorticoids (but not cAMP) induce desnutrin gene expression in 3T3-L1 cells. An increase in desnutrin mRNA was also observed when fully differentiated adipocytes were treated with dexamethasone (Fig. 5*B*). The increase did not appear to be as significant as that shown in preadipocytes, probably because of the high basal levels of desnutrin mRNA in adipocytes. The induction of desnutrin mRNA by dexamethasone was time- and dose-dependent (Fig. 5, *C* and *D*). Confluent preadipocytes treated for 48 h with increasing amounts of dexamethasone ranging from 1 nM to 10 μM showed detectable

levels of desnutrin mRNA at concentrations as low as 1 nM and a gradual increase in parallel with the dose of dexamethasone. The half-maximal response occurred in the nanomolar range, in agreement with the reported K_d for dexamethasone with the glucocorticoid receptor of 3T3-L1 cells (20), suggesting that the effect of dexamethasone on desnutrin mRNA levels was probably mediated by glucocorticoid binding to its receptor. Moreover, desnutrin expression was time-dependent, first detectable 24 h after dexamethasone treatment (Fig. 5*D*).

Intracellular Localization of EGFP-tagged Desnutrin Protein—PSORTII analysis of the deduced desnutrin amino acid sequence predicted a cytoplasmic localization of the protein. To determine whether the desnutrin protein is, in fact, located in the cytoplasm, we transiently transfected an expression vector for a desnutrin open reading frame-EGFP fusion protein into COS-7 cells and analyzed its localization by confocal microscopy. A C-terminal EGFP fusion protein was generated rather than an N-terminal EGFP fusion protein in an attempt to avoid potential masking of putative signal sequence. As shown in Fig. 6, desnutrin-EGFP showed a homogeneous distribution in the cells excluding the nucleus, suggesting a cytosolic localization of the desnutrin gene-encoded protein. On the other hand, in agreement with a previous report (18), examination of adiponutrin-EGFP expression showed a granular appearance consisting of punctate structures in the cytoplasm (data not shown).

To confirm the cytoplasmic localization of the desnutrin protein, COS-7 cells were transfected with an HA-tagged desnutrin expression vector, and crude subcellular fractionation followed by Western blot analysis was performed. HA-desnutrin was not detected in the mitochondrial or microsomal fraction, but a strong signal was detected in the cytosolic fraction (Fig. 6*B*). A strong signal was detected also in the nuclear fraction, but this probably was due to the presence of unbroken cells, as corroborated by optic microscopy (data not shown). Thus, we conclude that putative desnutrin is a cytoplasmic protein.

Effect of Ectopic Expression of Desnutrin on Triglyceride Hydrolysis—So far, no function has been attributed to any of
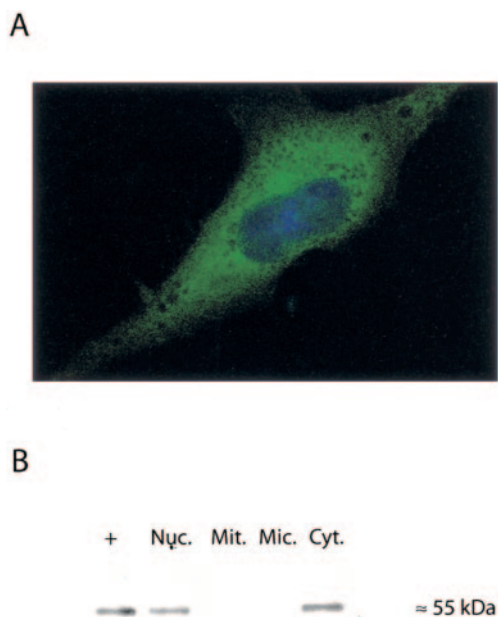
FIG. 6. **Subcellular localization of desnutrin-EGFP fusion protein.** *A*, COS-7 cells were transfected with the desnutrin-EGFP expression vector, and localization of the fusion protein was assessed by confocal microscopy. *B*, COS-7 cells were transfected with an HA-tagged desnutrin expression vector, and nuclear (*Nuc.*), mitochondrial (*Mit.*), microsomal (*Mic.*), and cytosolic (*Cyt.*) fractions were prepared as described under "Experimental Procedures." Five μg of protein from each fraction was subjected to SDS-PAGE, transferred to a polyvinylidene difluoride membrane, and analyzed for the presence of HA-desnutrin using anti-HA antibody. As a positive control for the immunodetection, 10 μg of whole cell lysate was used.

the desnutrin protein homologs in mammals. In an attempt to identify a potential functional domain in desnutrin, we compared its amino acid sequence with the Pfam protein family data base. A so-called patatin domain present in the region spanning amino acids 8–179 (Fig. 1) of desnutrin was identified. The domain owes its name to patatin, a storage protein found in plants that possesses lipid acyl hydrolase and broad esterase activity. Interestingly, ClustalW alignment of desnutrin and the related proteins described above showed that the highest degree of homology concentrates, precisely, in this patatin domain. Several regions conserved among the desnutrin-related proteins in metazoans could also be found in proteins of organisms as phylogenetically distant as plants, fungi, and prokaryotes (Fig. 7) (data not shown). Three highly conserved regions could be identified (Fig. 7): a glycine-rich G*X*G*XX*G nucleotide binding motif, a G*X*S*X*G serine hydrolase motif, and a D*X*(G/A) motif containing a conserved aspartate residue. The serine and aspartate residues constitute a catalytic dyad that is required for the lipase activity of patatin (21). The high homology between desnutrin, adiponutrin, TTS2, and GS2 indicates that these proteins belong to the same family, and the similarity in structure suggests a similar function.

The homology of the protein encoded by the desnutrin gene to the patatin-like protein family led us to investigate whether ectopic overexpression of desnutrin could affect hydrolysis of triglycerides due to its potential lipase activity. We labeled cellular lipid with [U-^{14}C]palmitic acid for 4 h in COS-7 cells expressing HA-desnutrin or control cells transfected with empty vector (Fig. 8*A*), and the lipid composition was analyzed by thin layer chromatography. As shown in Fig. 8*B*, after the labeling period (time 0), cells expressing desnutrin or cells transfected with a control empty vector showed the same lipid profile, and no quantitative difference among the different

types of lipids was observed between control and desnutrin-expressing cells. This indicates that synthesis of lipids was not affected by desnutrin overexpression. The labeled triglyceride levels did not change significantly when the control cells were maintained in normal medium for an additional 8 h. On the other hand, in desnutrin-overexpressing cells, the labeled triglyceride levels decreased significantly by ~40% (Fig. 8, *B*, *left panel*; and *D*, *lower panel*). There were no differences in the phospholipid and other lipid profiles (Fig. 8*B*, *right panel*), indicating that ectopic expression of HA-desnutrin affected only triglyceride hydrolysis. The reduction in labeled triglycerides observed in HA-desnutrin-transfected cells was time-dependent; and after 16 h of incubation, their levels in the cells were reduced further by 80% (Fig. 8*C*), whereas in control cells, there was only a 30% reduction in labeled triglycerides. These results clearly indicate that the triglycerides in desnutrin-overexpressing cells were hydrolyzed at a faster rate than in control cells.

The intracellular labeled free fatty acid levels decreased significantly during the 8-h period of incubation in both desnutrin-transfected and control cells. Therefore, despite the lower levels of labeled triglycerides detected in the desnutrin-transfected cells, we could not detect higher levels of intracellular free fatty acids, suggesting that the fatty acids resulting from triglyceride hydrolysis were either released to the medium or rapidly metabolized in the cell. We therefore examined the radiolabeled lipids in the culture medium of the COS cells (Fig. 8*D*, *upper panels*). TLC analysis of lipids extracted from the medium showed that the labeled lipids found in the medium were mainly free fatty acids (Fig. 8*D*, *upper left panel*). As shown in Fig. 8*D* (*upper right panel*), the labeled free fatty acids in the medium gradually increased when the cells were maintained in normal medium up to 8 h. Furthermore, desnutrin-expressing cells showed higher levels of radiolabeled free fatty acids released to the medium compared with control empty vector-transfected cells. Therefore, the decrease in labeled cellular triglyceride levels was accounted for in part by an increase in free fatty acids release to the medium, providing further evidence that the desnutrin-overexpressing cells have a higher rate of lipolysis.

DISCUSSION

In our search for genes that could play a crucial role in adipocyte differentiation or function, we previously have successfully used EST cDNA microarrays to identify genes that are expressed exclusively in adipose tissue (12). Using the same approach, we now have identified and cloned from adipose tissue a 1965-bp cDNA encoding a 486-amino acid putative protein that we have named desnutrin. The predicted amino acid sequence shows that desnutrin belongs to a distinct new family of proteins that have common structural features and that are regulated by the nutritional condition of the animals. So far, the genes encoding the two members of this family, desnutrin and adiponutrin, have been cloned and identified to be predominantly adipocyte-specific.

Whereas adiponutrin mRNA expression is restricted to adipose tissue, desnutrin mRNA, although expressed predominantly at a high level in adipose tissue, is also found at a low level in a variety of tissues. This favored expression in adipose tissue clearly suggests the involvement of the desnutrin gene in a function preferential to but not exclusive of adipose tissue. The increase in triglyceride hydrolysis associated with desnutrin overexpression and the induction of desnutrin gene expression by fasting are consistent with this hypothesis. Indeed, although most tissues use their triglycerides for hydrolysis and oxidation during energy depletion, the energy reserve is not sufficient, and these cells need to rely on the substrates pro-

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                            GXGXXG                                              GXSXG
TTS2.2      1   .........MFPREKTWNISFAGCGFLGVYYVGVASCLREHAPFLVANATHIYGASAGALTATALVTGVCLGEAGAKFIEVSKEARKRFLG
TTS2.1      1   .........MFPREKTWNISFAGCGFLGVYYVGVASCLREHAPFLVANATHIYGASAGALTATALVTGVCLGEAGAKFIEVSKEARKRFLG
Desnutrin   1   .........MFPRETKWNISFAGCGFLGVYHIGVASCLHEHAPFLVANATHIYGASAGALTATALVTGACLGEAGANIIEVSKEARKRFLG
CG5295-PA   1   ..............MNLSFAGCGFLGIYHVGVAVCFKKYAPHLLLEK..IGGGASAGSLAACCLLCDLPLGSMTSDFFRVVNEARRYSLG
C05D11.7    1   ......MTMINSRPELMNLSFSGCGFLCVYHAGVAAKIKEYAPHLLQNK..ILGASAGSIVACGLITGVCIGSHATSTILKVVSQARSRTFG
Adiponutrin 1   ......MYDPERRWSLSFAGCGFLGFYHVGATLCLSERAPHLLRDARTFFGCSAGALHAVTFVCSLPLGRIMEILMDLVRKARSRNIG
GS-2like    1   ........MGFLEEEGRWNLSFSGAGYLGAHHVGATECLRQRAPRLLQGARRIYGSSSGALNAVSIVCGKSVDFCCSHLLGMVGQLERLSLS
Atlg33270   100 QRVKDVEAEKERRVITSPGFSFSAAGLLFPYHLGVAQLLIEKG..YIKETTPLAGSSAGAIVCAVITSGATMREALEATKELAYDCRRN...

TTS2.2      83  PLHPSFNLVKIIRSFLLKVLEADSHEHASGRLGISLTRVSDG.ENVIISHFNSKDELIQANVCSGFIPVYCGLIPP.SLQGVRYVDGGISDN
TTS2.1      83  PLHPSFNLVKIIRSPLLKVLEADSHEHASGRLGISLTRVSDG.ENVIISHFNSKDELIQANVCSGFIPVYCGLIPP.SLQGVRYVDGGISDN
Desnutrin   83  PLHPSFNLVKTIRGCLLKTLEADCHERANGRLGISLTRVSDG.ENVIISHFSSKDELIQANVCSTFIPVYCGLIPP.TLQGVRYVDGGISDN
CG5295-PA   74  PFSPSFNIQTCLLEGLQKHLEDDAHKRVNGRLHISLTRVYDG.KNVIISEFESRENVILPVALLCACPIPLFSGLIPP.RFRGVRYMDGAFSDN
C05D11.7    84  PLHPEFNLLGIVRDELEVILFPNAYEMCTGRLVISLTRWSDH.ENVIIDEYRSNADLIDAIMCSCPIPLYCGITPP.KFRGVQYIDGGVSDN
Adiponutrin 83  TLHPFFNINKCIRDGLQESLEDNVHQVISGKVHISLTRVSDG.ENVLVSEFHSKDEVVDALVCSCFIPLFSGLIPP.SFRGERYVDGGVSDN
GS-2like    85  ILHPAYAPIEHVKQQLQDALFPDAHVLASQRLGISLTRWPDG.RNFLVTDFATCDELIQALVCTLYFFFYCGLIPP.EFRGERYIDGALSNN
Atlg33270   186 ..GTAFRLGAVLRESMERLLFDDIHIRSNGRIRVAITQVFWRPRGLLVDQFDSKSDLIDAVFTSSFIEGYLAPREATMFRNRLCVDGGLTLF

                                                                                     DXG/A
TTS2.2      173 LPLYELKNTITVSPFSGE......SDICPQDSST.NIHELRVTNTSIQFNLRNLYRLSKALFPPEPLVLREMCKQG...YRDGLRFLQRNGL
TTS2.1      173 LPLYELKNTITVSPFSGE......SDICPQDSST.NIHELRVTNTSIQFNLRNLYRLSKALFPPEPLVLREMCKQG...YRDGLRFLQ....
Desnutrin   173 LPLYELKNTITVSPFSGE......SDICPQDSST.NIHELRVTNTSIQFNLRNLYRLSKALFPPEPLVLREMCKQG...YRDGLRFLR....
CG5295-PA   164 LPILD.ENTITVSPFCGE......SDICPRDQSS.QLFHLNWANTSIEISRQNINRFVRILFPPRPEFLSKFCQQG...FDDALQFLHRNNL
C05D11.7    174 QPIYD.EHTVTVSPFSGE......SDICPDWDSGSMLGVDFNGTSIRFTTRNMFRLMACLWPRSTDDLSRMCLQG...FGBALRFL.....
Adiponutrin 173 VEVLDAKTTITVSPFYGE......HDICPKVKST.NFFHVNITNLSLRLCTGNLQLLTRALFPSDVKVMGELCYQG...YLDAFRFLE....
GS-2like    175 LPFADCPSTITVSPFHGT......VDICPQSTSP.NLHELNVFNFSFQISTENFFLGLICLIPPSLEVVADNCRQG...YLDALRF......
Atlg33270   277 MBPTAAAKTVRVCALSASNFKLKGIEICPDCNPLNRATSRQLLNWALEPAEDEVLERLFELGYADAATWAEMNPVEGLVYDGTPTAQEIPTS
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FIG. 7. **Multiple sequence alignment of amino acid sequences corresponding to the patatin domain of desnutrin and desnutrin-related proteins.** Alignment of desnutrin and desnutrin-related proteins revealed the presence of a highly conserved 180-amino acid N-terminal region identified as a patatin domain in the Pfam protein family data base. Alignment of the following sequences is shown: human TTS2.1, TTS2.2, and GS2-like protein; mouse desnutrin and adiponutrin; *D. melanogaster* CG5295-PA; *C. elegans* C05D11.7; and *A. thaliana* Atlg33270. Identical residues in all aligned sequences are indicated by *dark gray boxes*. Residues conserved in 80% of the aligned sequences are indicated by *light gray boxes*. The glycine-rich motif (G*X*G*XX*G), the active serine hydrolase motif (G*X*S*X*G), and the aspartate active site (D*X*(G/A)) are shown.

vided by adipose tissue, an organ specialized in energy storage in the form of triglycerides. It is worth noting that desnutrin mRNA levels vary depending on the adipose tissue type and depot. The differential regional expression of the desnutrin gene could be a factor contributing to the metabolic heterogeneity observed among different adipose depots (22, 23). Interestingly, desnutrin gene expression is lower in genetically *ob/ob* and *db/db* obese mouse models, suggesting a potential role of desnutrin in the pathophysiology of obesity.

The homology data from a BLASTp search did not provide clues to the potential role of desnutrin. On the other hand, a Pfam protein family data base search identified the presence of a patatin-like domain in the N-terminal region of desnutrin. Patatin is a member of a multigene family of proteins found in potato and other solanaceous plants (24). It accounts for 40% of the total soluble potato tuber protein and is considered to be a storage protein. In addition, patatin displays broad lipid acyl hydrolase activity (25, 26). This enzymatic activity relies on a catalytic dyad formed by a conserved serine residue in the G*X*S*X*G motif, characteristic of esterases, and a conserved aspartate residue belonging to the D*X*(G/A) motif (21). The structural resemblance of desnutrin to patatins, including the presence of the conserved residues that constitute the catalytic dyad, and the increase in triglyceride hydrolysis observed in cells overexpressing the desnutrin open reading frame support our hypothesis that the desnutrin protein may be a lipase.

When ectopically overexpressed, desnutrin seems to have effects specifically on triglycerides since no change in cholesterol or phospholipids was observed in the lipid labeling experiments. The 85-kDa calcium-independent phospholipase A$_2$, a mammalian protein with eight ankyrin motifs and a patatin domain with the serine-aspartate catalytic dyad, selectively hydrolyzes phospholipids at the *sn*-2 position (27). On the other hand, plant patatins act on a broad range of substrates, including phospholipids, glycolipids, sulfolipids, and mono- and diacylglycerols, but not triacylglycerols (25). Interestingly, ExoU, the *Pseudomonas aeruginosa* encoded type III cytotoxin, a recently described lipase containing a patatin domain, has triglycerides and other neutral lipids among its substrates (28). This disparity in substrate specificity, despite the high degree of conservation in the residues that form the catalytic domain,

suggests that the domain(s) responsible for substrate recognition may rely on regions that are less conserved among the different lipases containing a patatin domain. This differential specificity can also have important functional significance. The delivery of ExoU by *P. aeruginosa* is associated with lung injury and sepsis in animal models (29). These pathologies seem to be caused by alterations in the membrane permeability of the host cells due to the lipase activity of the toxin (28). In patatins, the broad lipase activity has been associated with a defense function, inhibiting the growth of some insect larvae by disrupting their mid-gut membranes (30). On the other hand, phospholipases A$_2$ specifically cleave the *sn*-2 ester bond of substrate phospholipids, and the released fatty acids can function as second messengers or precursors of eicosanoids that mediate signal transduction (31). The apparent specificity of the desnutrin gene-encoded protein for triglycerides, its tissue distribution, and the induction of expression by fasting suggest that the desnutrin gene could play a role in the response of the organism to starvation, enhancing hydrolysis of triglycerides and providing free fatty acids to other tissues to be oxidized in situations of energy depletion. In this regard, hormone-sensitive lipase is known to be a key enzyme in the mobilization of fatty acids from acylglycerols in adipocytes as well as, albeit low, in non-adipocytes (32). In hormone-sensitive lipase null mice, catecholamine-induced glycerol and fatty acid release is significantly blunted. However, significant levels of lipolysis occur in hormone-sensitive lipase null cells, and diglycerides accumulate in adipocytes, indicating the presence of additional lipase(s) that are not catecholamine-sensitive and that are mainly triglyceride lipases. Interestingly, as discussed below, desnutrin gene expression is regulated by glucocorticoids, but not by cAMP. We propose that desnutrin may function as a triglyceride lipase. However, because our present study was performed by transfection of the open reading frame of the desnutrin gene, examination of the endogenous protein using specific antibodies as well as RNA interference approaches in adipocytes will further clarify this conclusion.

The nutritional regulation of the murine patatin-like protein-encoding genes seems to be specific to each of the family members. Indeed, adiponutrin gene expression is abolished by fasting and up-regulated by feeding. The desnutrin gene, how-
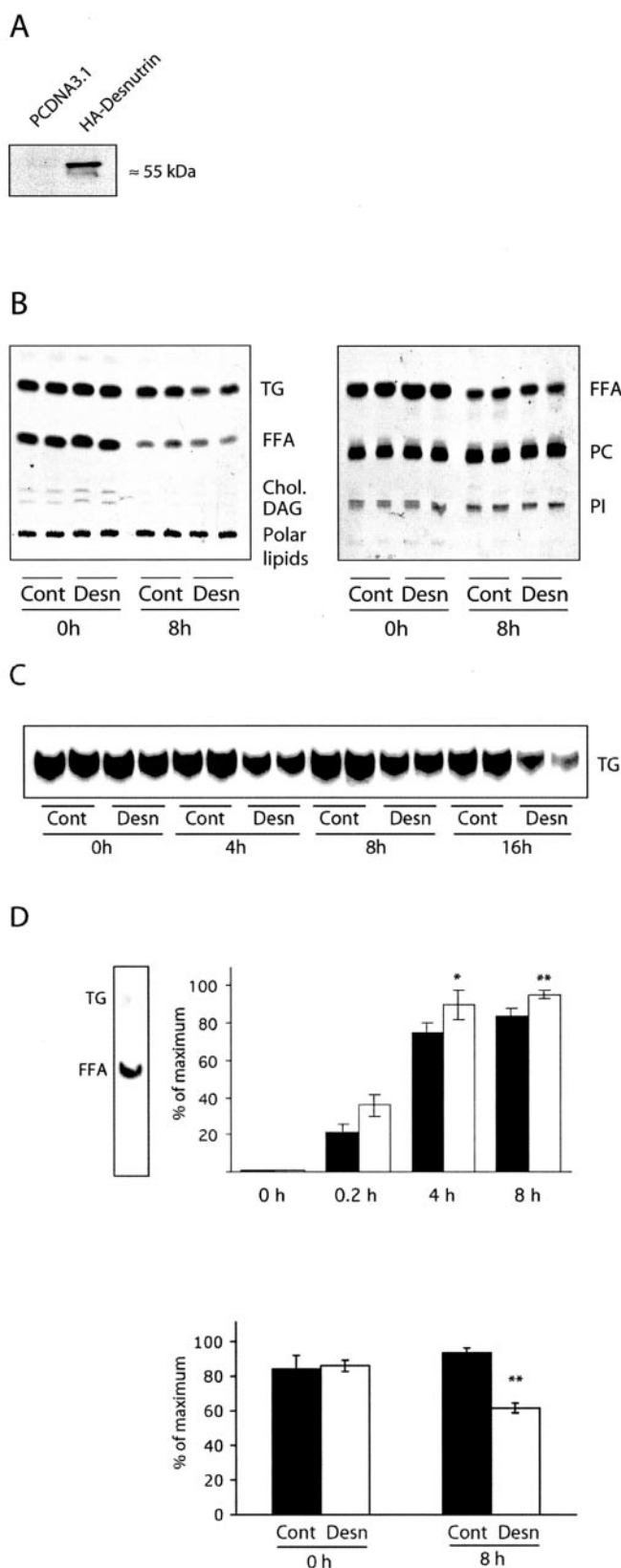
Adipose Desnutrin, a Patatin-like Protein, Increases Lipolysis

A

B

C

D

ever, shows an opposing pattern of expression, being transiently up-regulated by fasting. The opposite expression pattern of desnutrin and adiponutrin genes may reflect a differential hormonal regulation during fasting/feeding, and this suggests different functions for these proteins. We have demonstrated that desnutrin gene expression is induced by glucocorticoids, one of the hormones involved in the response of an organism to fasting, but not by cAMP. On the other hand, induction of adiponutrin gene expression by glucose has been reported to be counteracted by isoproterenol and forskolin, which mimic catecholamine action by raising the intracellular cAMP levels. The mode of action of glucocorticoids on desnutrin gene expression is still unclear. Undoubtedly, the fact that low concentrations of dexamethasone, close to the K_d for the glucocorticoid receptor, can induce desnutrin expression suggests that its effect is mediated by the glucocorticoid receptor. However, we have not found glucocorticoid response elements in the 5′-promoter region of the desnutrin gene. It is known that the glucocorticoid receptor can exert its effects not only by binding directly to the glucocorticoid response element, but also by interacting with other transcription factors such as activator protein-1 (33) and C/EBPβ (34) by a mechanism termed cross-coupling. Interestingly, several consensus binding sites for C/EBP that could mediate the glucocorticoid effect are present in the desnutrin promoter region (data not shown). Another possibility is an indirect action of glucocorticoids on desnutrin gene expression through an intermediary factor that is induced by glucocorticoids. This may explain the somewhat slow induction of desnutrin gene expression by dexamethasone. However, we did not observe any difference in desnutrin mRNA induction by dexamethasone in the presence of cycloheximide (data not shown).

In conclusion, with the cloning of desnutrin cDNA, we define a new family of mammalian genes coding for proteins characterized by the presence of a patatin-like domain as a structural feature. Expression of the two members of the family identified and characterized so far appears to be under strict hormonal and nutritional control. We suggest naming this gene family of desnutrin homologs and the protein they encode "nutrins," given the control nutritional status exerts on its expression in adipose tissue. Both desnutrin and adiponutrin genes are highly expressed in adipose tissue, and desnutrin protein activity seems to be involved in triglyceride hydrolysis and, by extension, in energy homeostasis. In this regard, genetic obesity models show lower levels of desnutrin expression, suggesting its potential contribution to the pathophysiology of obesity. However, *in vitro* biochemical studies, including characterization of the endogenous protein, are needed for definitive evidence for the function of desnutrin and adiponutrin. It would be interesting to see whether desnutrin, which we found localized in the cytoplasm, could be translocated to lipid droplets, which could be regulated by perilipin, as in the case of hormone-sensitive lipase (35). Furthermore, *in vivo* experiments on the

TLC autora-diograms using solvent system A to separate neutral lipids and free fatty acids (*FFA*) (*left panel*) and solvent system B to separate phospholipids and free fatty acids (*right panel*). *TG*, triglycerides; *Chol.*, cholesterol; *DAG*, diacylglycerol; *PC*, phosphatidylcholine; *PI*, phosphatidylinositol. *C*, shown are the results from time course analysis of labeled triglyceride content in control and HA-desnutrin-transfected COS-7 cells. Labeled lipids were isolated, resolved by TLC, and detected by autoradiography. Bands corresponding to triglycerides are shown. *D*, labeled lipids in the cell culture medium corresponded mostly to free fatty acids as determined by TLC (*upper left panel*). Labeled lipids in the cell culture medium, mostly FFA, were quantified in a scintillation counter (*upper right panel*). Densitometric scanning of intracellular triglycerides from two independent experiments was also performed (*lower panel*). Results are expressed as the means ± S.E. *, $p < 0.05$; **, $p < 0.01$.

FIG. 8. **Effect of ectopic expression of desnutrin on hydrolysis of triglycerides in COS-7 cells.** COS-7 cells transfected with pcDNA3.1 (control (*Cont*)) or the HA-desnutrin expression vector (*Desn*) were labeled with [U-^{14}C]palmitic acid for 4 h. After washing, cells were maintained in serum-containing medium. At this time, cells were collected (time 0) or incubated for an additional 8 h before harvesting. Total lipids were extracted, and lipid composition was analyzed by TLC using two solvent systems. *A*, shown is desnutrin expression in COS-7 cells after transient transfection with the HA-tagged desnutrin expression vector or the empty pcDNA3.1 vector. *B*, shown are representative

gain or loss of function using transgenic technology will certainly clarify the role of desnutrin in the response of the organism to caloric shortage as well as its possible implication in pathologies associated with altered adipose function or lipid metabolism such as obesity, lipodystrophy, and diabetes.

REFERENCES

1. Rosen, E. D., and Spiegelman, B. M. (2000) *Annu. Rev. Cell Dev. Biol.* **16,** 145–171
2. Gregoire, F. M., Smas, C. M., and Sul, H. S. (1998) *Physiol. Rev.* **78,** 783–809
3. MacDougald, O. A., and Mandrup, S. (2002) *Trends Endocrinol. Metab.* **13,** 5–11
4. Fruhbeck, G., Gomez-Ambrosi, J., Muruzabal, F. J., and Burrell, M. A. (2001) *Am. J. Physiol.* **280,** E827–E847
5. Unger, R. H., and Orci, L. (2001) *FASEB J.* **15,** 312–321
6. Burton, G. R., Nagarajan, R., Peterson, C. A., and McGehee, R. E., Jr. (2004) *Gene (Amst.)* **329,** 167–185
7. Guo, X., and Liao, K. (2000) *Gene (Amst.)* **251,** 45–53
8. Sadowski, H. B., Wheeler, T. T., and Young, D. A. (1992) *J. Biol. Chem.* **267,** 4722–4731
9. Smas, C. M., and Sul, H. S. (1993) *Cell* **73,** 725–734
10. Zhao, L., Gregoire, F., and Sul, H. S. (2000) *J. Biol. Chem.* **275,** 16845–16850
11. Villena, J. A., Kim, K.-H., and Sul, H. S. (2002) *Horm. Metab. Res.* **34,** 664–670
12. Kim, K.-H., Lee, K., Moon, Y. S., and Sul, H. S. (2001) *J. Biol. Chem.* **276,** 11252–11256
13. Kim, K.-H., Zhao, L., Moon, Y. S., Kang, C., and Sul, H. S. (2004) *Proc. Natl. Sci. U. S. A.* **101,** 6780–6785
14. Banerjee, R. R., Rangwala, S. M., Shapiro, J. S., Rich, A. S., Rhoades, B., Qi, Y., Wang, J., Rajala, M. W., Pocai, A., Scherer, P. E., Steppan, C. M., Ahima, R. S., Obici, S., Rossetti, L., and Lazar, M. A. (2004) *Science* **303,** 1195–1198
15. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) *Nucleic Acids Res.* **25,** 3389–3402
16. Gulick, T. (1996) in *Current Protocols in Molecular Biology* (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K., eds) John Wiley & Sons, Inc., New York
17. Bligh, E. G., and Dyer, W. J. (1959) *Can. J. Med. Sci.* **37,** 911–917
18. Baulande, S., Lasnier, F., Lucas, M., and Pairault, J. (2001) *J. Biol. Chem.* **276,** 33336–33344
19. Sul, H. S., and Wang, D. (1998) *Annu. Rev. Nutr.* **18,** 331–351
20. Nakada, M. T., Stadel, J. M., Poksay, K. S., and Crooke, S. T. (1987) *Mol. Pharmacol.* **31,** 377–384
21. Rydel, T. J., Williams, J. M., Krieger, E., Moshiri, F., Stallings, W. C., Brown, S. M., Pershing, J. C., Purcell, J. P., and Alibhai, M. F. (2003) *Biochemistry* **42,** 6696–6708
22. Martin, M. L., and Jensen, M. D. (1991) *J. Clin. Investig.* **88,** 609–613
23. Wahrenberg, H., Lonnqvist, F., and Arner, P. (1989) *J. Clin. Investig.* **84,** 458–467
24. Ganal, M. W., Bonierbale, M. W., Roeder, M. S., Park, W. D., and Tanksley, S. D. (1991) *Mol. Gen. Genet.* **225,** 501–509
25. Hirschberg, H. J., Simons, J. W., Dekker, N., and Egmond, M. R. (2001) *Eur. J. Biochem.* **268,** 5037–5044
26. Andrews, D. L., Beames, B., Summers, M. D., and Park, W. D. (1988) *Biochem. J.* **252,** 199–206
27. Tang, J., Kriz, R. W., Wolfman, N., Shaffer, M., Seehra, J., and Jones, S. S. (1997) *J. Biol. Chem.* **272,** 8567–8575
28. Sato, H., Frank, D. W., Hillard, C. J., Feix, J. B., Pankhaniya, R. R., Moriyama, K., Finck-Barbancon, V., Buchaklian, A., Lei, M., Long, R. M., Wiener-Kronish, J., and Sawa, T. (2003) *EMBO J.* **22,** 2959–2969
29. Allewelt, M., Coleman, F. T., Grout, M., Priebe, G. P., and Pier, G. B. (2000) *Infect. Immun.* **68,** 3998–4004
30. Strickland, J. A., Orr, G. L., and Walsh, T. A. (1995) *Plant Physiol.* **109,** 667–674
31. Six, D. A., and Dennis, E. A. (2000) *Biochim. Biophys. Acta* **1488,** 1–19
32. Holm, C. (2003) *Biochem. Soc. Trans.* **31,** 1120–1124
33. Schule, R., Rangarajan, P., Kliewer, S., Ransone, L. J., Bolado, J., Yang, N., Verma, I. M., and Evans, R. M. (1990) *Cell* **62,** 1217–1226
34. Nishio, Y., Isshiki, H., Kishimoto, T., and Akira, S. (1993) *Mol. Cell. Biol.* **13,** 1854–1862
35. Sztalryd, C., Xu, G., Dorward, H., Tansey, J. T., Contreras, J. A., Kimmel, A. R., and Londos, C. (2003) *J. Cell Biol.* **161,** 1093–1103

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