Transcriptional regulation by the $\gamma 5$ subunit of a heterotrimeric G protein during adipogenesis

Jin-Gyoon Park, Aleixo Muise,
Gong-Ping He¹, Sung-Woo Kim and
Hyo-Sung Ro²

Department of Biochemistry and Molecular Biology, Faculty of Medicine, Sir Charles Tupper Medical Building, Dalhousie University, Halifax, Nova Scotia, B3H 4H7, Canada

¹Present address: Myriad Genetics Inc., 390 Wakara Way, Salt Lake City, UT 84108, USA

²Corresponding author.
e-mail: hсро@is.dal.ca

J.-G.Park and A.Muise contributed equally to this work

The adipocyte enhancer-binding protein (AEBP1) is a novel transcriptional repressor with carboxypeptidase activity. A two-hybrid screen was conducted to identify components of AEBP1 that might be important in regulating its activity. The $\gamma 5$ subunit of a heterotrimeric G protein was shown to bind specifically to AEBP1 and to attenuate its transcriptional repression activity. Adipogenic stimulation selectively decreased the $\text{G}\gamma 5$ level and enhanced the transcriptional repression activity of AEBP1 during mitotic clonal expansion at the onset of adipogenesis. Thus, the actions of $\text{G}\gamma 5$ and AEBP1 are directly linked, which could provide the basis for the regulation of transcription at the onset of differentiation. This report shows that a signal-transducing molecule is involved, by direct protein–protein interaction, in the regulation of transcription during adipogenesis.

Keywords: adipogenesis/AEBP1/heterotrimeric G protein/transcriptional regulation

Introduction

The stimulation of adipose differentiation is characterized by an increased lipogenic capacity, a change from a fibroblast morphology to the unilocular appearance of mature adipocytes and an alteration in the expression levels of hundreds of proteins (for a review, see Gregoire *et al.*, 1998), including the adipocyte P2 gene (*aP2*) that encodes the adipocyte fatty acid-binding protein (Hunt *et al.*, 1986; Matarese and Bernlohr, 1988). In the *aP2* promoter region, the AE-1 sequence (nucleotides –159 to –125) functions as an enhancer element in the regulation of *aP2* expression (Distel *et al.*, 1987; Herrera *et al.*, 1989), and *trans*-acting factors, including the adipogenic transactivator C/EBP α (Christy *et al.*, 1989; Herrera *et al.*, 1989; Cao *et al.*, 1991), human pre-adipocyte factors (Ro and Roncari, 1991) and the transcriptional repressor adipocyte enhancer-binding protein 1 (AEBP1) (He *et al.*, 1995), have been defined. AEBP1 is a novel carboxypeptidase (CP), and its CP activity is vital for the tran-

scriptional repression function. AEBP1 expression is down-regulated during adipocyte differentiation (He *et al.*, 1995).

To understand further the physiological role of AEBP1, we have used a two-hybrid screen (Chien *et al.*, 1991; Durfee *et al.*, 1993; Harper *et al.*, 1993) to search for proteins that interact with AEBP1. These yeast two-hybrid studies revealed that AEBP1 interacts directly with the $\gamma 5$ subunit (Fisher and Aronson, 1992) of a heterotrimeric G protein. Heterotrimeric G proteins are important mediators of signal transduction in eukaryotic cells, linking ligand-bound seven-transmembrane receptors (also called G protein-coupled receptors) with the intracellular machinery such that signals delivered extracellularly result in an appropriate cellular response (for a review see Clapham and Neer, 1997). Upon receptor activation by an extracellular signal and interaction of the triggered receptors with G proteins, the α subunit releases GDP in exchange for GTP. The trimer then dissociates into α and $\beta\gamma$ subunits that can interact independently with effectors. The $\text{G}\beta\gamma$ dimer plays a major part in signal transmission (for a review see Clapham and Neer, 1997). The physiological role of AEBP1 in this signal transduction presently is unknown.

Here we describe co-immunoprecipitation, cell fractionation and immunofluorescent experiments which demonstrate that AEBP1 complexes with $\text{G}\gamma 5$ and $\text{G}\beta$, and that the G proteins localize to the nucleus in 3T3-L1 but not in NIH 3T3 cells. We also have used ectopically expressed $\text{G}\gamma 5$ or recombinant $\text{G}\gamma 5$ protein to demonstrate that $\text{G}\gamma 5$ attenuates the transcriptional repression activity of AEBP1 in transiently transfected cells or in an *in vitro* transcription run-off assay. Finally, we show that adipogenic stimulation selectively reduces the abundance of $\text{G}\gamma 5$ and enhances the repression activity of AEBP1 during the mitotic clonal expansion stage of adipogenesis. Hence, the actions of $\text{G}\gamma 5$ and AEBP1 are directly linked, which could provide the basis for the regulation of transcription at the onset of differentiation.

Results

AEBP1 complexes with the $\gamma 5$ and β subunits of a heterotrimeric G protein

We used co-immunoprecipitation to establish that AEBP1 and $\text{G}\gamma 5$ associate in mammalian cells. Cell extracts were subjected to immunoprecipitation with antibody to either AEBP1 or $\text{G}\gamma 5$, and each immunoprecipitate was separated by SDS–PAGE and probed with antibody to either AEBP1 or $\text{G}\gamma 5$. As shown in Figure 1A (top panel), AEBP1 was detected in the $\text{G}\gamma 5$ immunoprecipitates (lane 2) as well as in the AEBP1 immunoprecipitate (lane 1). No such protein band was detected in the control immunoprecipitate (lane 4). The immunoprecipitated samples were also

Fig. 1. AEBP1 complexes with the γ 5 and β subunits of a heterotrimeric G protein. (A) AEBP1 co-immunoprecipitates with G γ 5 and G β . Cell extracts were immunoprecipitated with antibodies to either AEBP1 (lane 1), G γ 5 (lane 2) or G β (lane 3), or with normal IgG (lane 4). The immunoprecipitates were resolved electrophoretically and probed with antibodies to AEBP1 (top panel), G β (middle panel) and G γ 5 (bottom panel). (B) G γ 5 interacts specifically with AEBP1. COS-7 cells were transfected with 20 μ g of either pJ3H-AEBP1(-) (lanes 1–5) or pJ3H-AEBP1 (lanes 6–10). Cell extracts were immunoprecipitated with antibodies to HA (lanes 1 and 6), G γ 5 (lanes 2 and 7), G γ 7 (lanes 3 and 8), G β (lanes 4 and 9) and IGFR (lanes 5 and 10). The immunoprecipitates were immunoblotted with anti-HA antibody. The anti-G β antibody, G β (T-20), that is broadly reactive with G β 1, G β 2, G β 3 and G β 4 (Santa Cruz Biotechnology) was used. Antibodies to G γ 7 and the insulin-like growth factor receptor (IGFR) were used as negative controls. Antibodies to cAMP-dependent protein kinase (PKA α) and a small subunit ribosomal protein S24 were also used as negative controls (data not shown).

analyzed by immunoblotting with anti-G γ 5 antibody. As shown in Figure 1A (bottom panel), G γ 5 was detected in the AEBP1 immunoprecipitate (lane 1) as well as in the G γ 5 immunoprecipitate (lane 2). No such protein band was detected in the control immunoprecipitate (lane 4).

To determine further whether AEBP1 pulls down G γ 5 specifically, extracts from cells transfected with an expression plasmid (pJ3H-AEBP1) encoding a hemagglutinin (HA)-tagged version of AEBP1 (HA-AEBP1) were subjected to immunoprecipitation with antibody directed against HA, G γ 5 or the related γ subunit G γ 7, and each immunoprecipitate was analyzed as above. As shown in Figure 1B, HA-AEBP1 was detected in the G γ 5 immunoprecipitate (lane 7), but not in the G γ 7 immunoprecipitate (lane 8). HA-AEBP1 was not detected in the G γ 5 immunoprecipitate from cells transfected with the control plasmid pJ3H-AEBP1(-) (lane 2). Moreover, no HA-AEBP1 was detected in control (anti-IGFR) immunoprecipitates from cells transfected with either pJ3H-AEBP1 (lane 10) or pJ3H-AEBP1(-) (lane 5). These results strongly suggest that AEBP1 and the γ 5 subunit are associated in mammalian cells. The interaction of AEBP1

with G γ 5 is specific since AEBP1 was not co-precipitated with G γ 7 (lane 8), which implies that the lipid moiety normally present on G γ subunits may not be responsible for the interaction.

Since G protein β and γ subunits are thought to be tightly associated *in vivo* and their regulatory effects are ascribed to the intact dimer (for a review see Clapham and Neer 1997), we examined whether G β is also present in the AEBP1–G γ 5 complex. Indeed, AEBP1 was detected in the G β immunoprecipitate (Figure 1A, top panel, lane 3). Similarly, HA-AEBP1 was detected in the G β immunoprecipitate from cells transfected with pJ3H-AEBP1 (Figure 1B, lane 9), but not that from cells transfected with pJ3H-AEBP1(-) (lane 4). Conversely, G β was detected in the AEBP1 immunoprecipitate (Figure 1A, middle panel, lane 1) as well as in the G β immunoprecipitate (lane 3). As expected, G β was detected in the G γ 5 immunoprecipitate (lane 2). No such protein band was detected in the control immunoprecipitate (lane 4). Thus, in mammalian cells, AEBP1 and the G β γ 5 dimer may form a ternary complex. We do not exclude the possibility that other proteins associate in such a complex, or that smaller complexes of subsets of these proteins can form.

The G γ 5 and G β subunits localize in the nucleus in 3T3-L1 cells

The AEBP1–G γ 5 interaction may occur only when G γ 5 localizes in the nucleus or when AEBP1 is associated with the plasma membrane. G γ subunits are found mainly at the plasma membrane, where they remain associated by integration of the lipid moiety into the phospholipid bilayer. All 10 mammalian γ subunits, γ 1, γ 2, γ 3, γ 4, γ 7, two γ 8s, γ 10, γ 11 and γ 12, have the C-terminal CAAX motif (where C = cysteine, A = aliphatic amino acid, and X = any amino acid), which is a signal for the post-translational modification (prenylation and carboxyl-methylation) of the cysteine residue. The three C-terminal residues are removed proteolytically, and the carboxyl group of the resulting prenylcysteine is carboxyl-methyl esterified (for a review see Zhang and Casey, 1996). G γ 5 is unique in that it has a non-aliphatic serine and an aromatic amino acid phenylalanine in the CAAX motif (CSFL). Recently, an alternative processing pattern, which retains the three terminal amino acids, for the γ 5 subunit has been identified (Cook *et al.*, 1998). This alternative pattern of processing might explain the unique association of γ 5 with focal adhesion plaques in neonatal cardiac fibroblasts (Hansen *et al.*, 1994) and in Swiss 3T3 and C6 glioma cells (Ueda *et al.*, 1997). In yeast, disruption of the gene coding for the protease that removes the three C-terminal amino acids of prenylated proteins results in the redistribution of Ras2p to internal membranes (Boyartchuk *et al.*, 1997). Therefore, it is possible that γ 5 targets to different locations in the cell.

Since the nuclear and plasma membrane fractions were of major interest in this study, we performed subcellular fractionation in which nuclear (N), post-nuclear soluble (S) and particulate (PP) [which contains mainly the plasma membrane rather than other membrane compartments, e.g. the endoplasmic reticulum (ER), lysosome and endosomes] fractions were prepared and analyzed by immunoblotting. Two isoforms of the acetyl-CoA carboxylase (of 220 and 240 kDa), a cytosolic protein, are seen as one band on an

Fig. 2. The G γ 5 and G β subunits localize in the nucleus in 3T3-L1 cells. **(A)** 3T3-L1 cells were fractionated into nuclear (N), post-nuclear soluble (S) and particulate (PP) fractions, and proteins in each fraction from the same number of cells were analyzed by Western blotting. Acetyl-CoA carboxylase (AC), a biotinylated cytosolic protein, was detected with HRP-conjugated avidin and used to estimate the contamination of unbroken cells in the nuclear fraction. IGFR and its precursor form (pIGFR) were used as the plasma membrane markers. c-Myc and a general transcription factor, TFIIB, were used as the nuclear markers. Cells were starved in serum-free medium for 24 h and analyzed for c-Myc. **(B)** 3T3-L1 cells were fractionated as in (A) and analyzed by Western blotting for AEBP1, G β , G γ 5 and G γ 7. **(C)** NIH 3T3 cells were fractionated as in (A) and analyzed by Western blotting for G β , G γ 5 and G γ 7. **(D)** Immunofluorescent confocal laser microscopy shows the subcellular distribution of G γ 5 (A and B), G γ 7 (C and D) and G β (E and F) in 3T3-L1 (A, C and E) and NIH 3T3 (B, D and F) cells. Panels G and H show immunofluorescence with normal IgG. All pictures were taken at the same magnification ($\times 400$), and represent the nuclear sections at 3 μ m from the bottom of the cells. The length of the scale bars is 50 μ m. Higher magnification allowed a better estimate of the plasma membrane localization of all the G protein subunits (data not shown).

8.5% polyacrylamide gel, and the band can be seen almost exclusively in the S, but not in the N or PP, fraction (Figure 2A, top panel). Thus, the N fraction is largely free of contamination with intact cells or debris. As shown in Figure 2A, c-Myc, a transcription factor, was localized exclusively in the N fraction. TFIIB, a general transcription factor, was also present mainly in the nuclear fraction, while a lesser amount ($<10\%$ of total signal) was observed in other fractions. On polyacrylamide gels, insulin-like growth factor receptor (IGFR), a transmembrane protein, migrates as a 100 kDa band, and a precursor of the receptor (pIGFR) is also detected above 200 kDa. As shown in Figure 2A, these two forms of IGFR were

primarily in the PP fraction. A band with an apparent mol. wt of ~ 83 kDa in the N fraction may represent a non-specific protein or an immunologically related protein. Therefore, our fractionation procedure is satisfactory.

To determine the subcellular distribution of AEBP1 and G γ 5, the amount of protein representing the cell equivalent of each fraction was analyzed by Western blotting. By this analysis, AEBP1 was observed at similar levels in the N and S fractions prepared from the pre-adipocyte cell line 3T3-L1 (Figure 2B). The AEBP1 band in the N fraction migrated slightly more slowly than that in the S fraction, which suggests that AEBP1 is modified post-translationally and that the modification may determine the

localization of AEBP1. G γ subunits are expected to localize to the plasma membrane, i.e. in the PP fraction. Since G γ 5 and G γ 7 are the major G γ subunits in fibroblasts such as 3T3-L1 cells (Ueda *et al.*, 1997), the localization patterns of these two G γ subunits were determined. As expected, G γ 7 was localized almost exclusively to the PP fraction. In contrast, a substantial amount of G γ 5 was detected in the N fraction (Figure 2B). This is in sharp contrast to the pattern in NIH 3T3 cells, a mouse embryonic fibroblastic cell line that is unable to differentiate to adipocytes, in which G γ 5 was localized mainly to the PP fraction (Figure 2C). This finding indicates that G γ 5 specifically localizes to the nucleus in 3T3-L1 cells, where it potentially could interact with AEBP1, or that G γ 5 may be transported to the nucleus in a complex with AEBP1. Since G protein β and γ subunits are thought to be tightly associated *in vivo*, it is likely that G γ 5 and G β localize together in the nucleus. Since G γ 5 is the most abundant G γ subunit in fibroblasts, it is assumed that the majority of G β subunits would form dimers with G γ 5 and that their subcellular distribution patterns would be similar. Indeed, similar subcellular distributions of G β and G γ 5 were observed (Figure 2B). Since the G β antibody was raised against a peptide common to all six known G β subunits, the band probably represents a pool of G β subunits that interact with G γ 5. A weaker band was seen in the S fraction, which may represent G β in the ER and/or Golgi apparatus, or contamination from other fractions. Upon longer exposure, a smaller amount of G γ 5 was also seen in the S fraction. The distribution pattern in NIH 3T3 cells showed that both G β and G γ 5 subunits are localized mainly to the PP fraction (Figure 2C).

Confocal laser microscopic analysis (Hansen *et al.*, 1994) confirmed the nuclear localization of G γ 5 and G β in 3T3-L1 cells (Figure 2D, panels A and E). Consistent with the cell fractionation data, G γ 5 and G β were not detected in the nuclei of NIH 3T3 cells (panels B and F). As in the case of the cell fractionation analysis, G γ 7 was also not detected in the nuclei of either cell type (panels C and D). Therefore, it appears that the nuclear localization of the G protein subunits is cell type specific. Nuclear localization of several G α (e.g. G α s, G α 2 and G α 3) and G β subunits has been reported (Crouch, 1991; Bégin-Heick *et al.*, 1997; Crouch and Simson, 1997). The physiological significance of the nuclear localization of these G protein subunits is not clearly understood. The nuclear localization of G γ 5 in certain cell types implies a specific physiological role for G γ 5 in such cell types.

G γ 5 regulates transcriptional repression activity of AEBP1

We investigated the biological significance of the association between G γ 5 and AEBP1 by determining AEBP1 transcriptional repression activity *in vivo*. We have shown previously that the reporter plasmid paP2(–168)CAT, which contains the *aP2* promoter region (–168 to +21) fused to the reporter gene chloramphenicol acetyltransferase (CAT), could be used to assess AEBP1 transcriptional repression activity through its binding site (AE-1) in the proximal promoter region. When an expression plasmid encoding AEBP1 (pSVAEBP1) was co-transfected with paP2(–168)CAT, CAT activity was significantly decreased (He *et al.*, 1995). We transfected an expression

plasmid encoding G γ 5 (pRc/CMV γ 5) along with both pSVAEBP1 and paP2(–168)CAT, and determined the repression activity of AEBP1 (He *et al.*, 1995). As shown in Figure 3A, the repression activity of AEBP1 was attenuated by ectopic overexpression of G γ 5, and this inhibition by G γ 5 of repression activity was dose responsive. Transfection of a plasmid containing the G γ 5 cDNA in the opposite orientation [pRc/CMV γ 5(–)] along with the two aforementioned plasmids had no effect on CAT expression (Figure 3A). Co-transfection of pRc/CMV γ 5 with paP2(–168)CAT alone had no effect on CAT expression (data not shown). Therefore, the inhibition by G γ 5 of repression activity was not due to non-specific up-regulation of CAT expression.

Since it was unclear whether G γ 5 inhibits AEBP1 repression activity directly or via intermediates, we developed an *in vitro* transcription system utilizing bacterially produced recombinant proteins. The transcription template pCMV/AE-1 contains a human cytomegalovirus (CMV) gene fragment (–829 to +363) with three copies of the AE-1 sequence inserted upstream of the CMV promoter (Figure 3B). We tested transcription activity by using an *in vitro* run-off transcription assay utilizing HeLa cell nuclear extracts. When recombinant AEBP1 was added to the nuclear run-off transcription reaction along with the pCMV/AE-1 template, transcription activity was significantly decreased (Figure 3C, lanes 1 and 2), but when tested with the template pCMV, without AEBP1-binding sites, recombinant AEBP1 protein had no effect on transcription (lanes 3 and 4). These results demonstrate that, as seen *in vivo* (He *et al.*, 1995), the repression activity of AEBP1 requires localization to a specific promoter region, and is not due to transcriptional ‘squenching’ or to a non-specific shutdown of the RNA polymerase II machinery. Next, we tested whether recombinant G γ 5 protein, which would not have the lipid moiety, has the ability to inhibit the transcriptional repression activity of AEBP1. When recombinant G γ 5 protein was added to the nuclear run-off reaction, the repression activity of AEBP1 was virtually abolished (Figure 3D, lanes 4–6). This derepression effect of recombinant G γ 5 protein was not due to a non-specific transcriptional enhancement, since the transcription level did not increase when the recombinant G γ 5 protein was added to a transcription reaction with the pCMV/AE-1 template but without AEBP1 (lane 3). Furthermore, the recombinant G γ 5 protein had no significant effect on transcription of the pCMV template (lanes 7–10). Therefore, these results demonstrate that the lipid moiety may not be necessary for the inhibitory interaction, and that G γ 5 may inhibit the repression activity of AEBP1 directly.

Since AEBP1 binding to the AE-1 sequence is crucial for transcriptional repression by AEBP1, we determined whether the effect of G γ 5 was due to an interaction with AEBP1 that prevents binding to the AE-1 site. This possibility was examined by electrophoretic mobility shift assays (EMSAs). We have shown using EMSAs that recombinant AEBP1 protein specifically binds the AE-1 sequence (He *et al.*, 1995). As shown in Figure 3E, the recombinant G γ 5 protein was able to prevent binding of recombinant AEBP1 protein to the AE-1 sequence (lanes 10–12). A control recombinant protein had no effect on DNA binding by AEBP1 (lanes 2–4). Moreover, the

Fig. 3. Gγ5 regulates transcriptional repression activity of AEBP1. (A) Gγ5 inhibits AEBP1 transcriptional repression activity. NIH 3T3 cells were transiently transfected with 2.5 μg of pA2(-168)CAT along with 5 μg of either pSVAEBP1(-) or pSVAEBP1. The repression activity of pSVAEBP1 on CAT expression was decreased in a dose-dependent manner when cells were co-transfected with increasing amounts (0.5, 1, 2 and 5 μg) of the γ5 expression plasmid pRC/CMVγ5, but not with the control plasmid pRC/CMVγ5(-). The values are averages from three separate transfection experiments. (B) Schematic representation of the *in vitro* transcription templates for nuclear run-off experiments. The nuclear run-off reaction would produce a 363 bp transcript. (C) Nuclear run-off reaction using either pCMV/AE-1 (lanes 1 and 2) or pCMV (lanes 3 and 4) templates without (lanes 1 and 3) or with (lanes 2 and 4) 200 ng of recombinant AEBP1 protein. (D) Gγ5 directly inhibits AEBP1 transcriptional repression activity. Nuclear run-off reaction using either pCMV/AE-1 (lanes 1-6) or pCMV (lanes 7-10) templates alone (lanes 1 and 7), with 200 ng of either AEBP1 (lanes 2 and 8) or γ5 (lanes 3 and 9), or with 200 ng of AEBP1 plus 50 (lane 4), 100 (lane 5) and 200 ng (lanes 6 and 10) of γ5. (E) Gγ5 directly interacts with AEBP1 and prevents binding of AEBP1 to AE-1 oligonucleotides. Recombinant AEBP1 protein was incubated with AE-1 oligonucleotides and a mobility shift was observed. Lane 1, 2 μg of AEBP1 plus 2 μg of BSA; lanes 2-4, 2 μg of AEBP1 plus 0.5, 1 and 2 μg of control (recombinant Rab11) protein, respectively; lane 5, 2 μg of Rab11 alone; lane 6, without any protein (probe alone); lane 7, 2 μg of γ5 alone; lane 8, 2 μg of AEBP1 plus unlabeled AE-1 oligonucleotides; lane 9, 2 μg of AEBP1 alone; lanes 10-12, 2 μg of AEBP1 plus 0.5, 1 and 2 μg of γ5.

recombinant Gγ5 protein had no effect on DNA binding by other transcription factors (data not shown). Thus, Gγ5 may regulate transcriptional repression by AEBP1 by preventing the tethering of AEBP1 to the promoter. The Gγ5 protein may directly mask the DNA-binding domain of AEBP1; alternatively, the interaction may cause a conformational change in AEBP1 which renders it unable to bind the AE-1 sequence.

Gγ5 level decreases selectively at the onset of adipogenesis

The above results demonstrated that Gγ5 can regulate AEBP1 transcriptional repression activity. We next asked whether the actions of Gγ5 are biologically relevant in adipogenesis. Adipogenesis is a complex process affected, in a positive or negative manner, by various hormones

and growth factors (for a review see Gregoire *et al.*, 1998). G proteins also play a prominent role in adipogenesis. The levels of Gα and Gα2, the G proteins that mediate activation and inhibition of adenylyl cyclase activity respectively, have been shown to modulate the rate of adipogenesis in 3T3-L1 cells (Wang *et al.*, 1992; Su *et al.*, 1993). These studies indicated that Gα and Gα2 are acting in a counter-regulatory manner, and that they mediate adipocyte differentiation in a manner apparently independent of adenylyl cyclase. Since these G proteins are localized to the nucleus in response to growth factors and hormones and play a prominent role in adipogenesis, one exciting possibility is that Gγ5 may have a direct physiological role in adipogenesis through its actions. To examine this possibility, we determined the subcellular distribution pattern of γ5 and β subunits upon adipogenic

Fig. 4. Adipogenic inducers selectively reduce the abundance of Gγ5 and enhance the repression activity of AEBP1 during mitotic clonal expansion at the onset of adipogenesis. (A) Confluent 3T3-L1 cells (day 0) were subjected to the differentiation protocol and cell extracts were prepared (at the times indicated), and equal amounts (10 μg) of the lysates were analyzed by Western blotting with anti-Gγ5 or anti-Gβ antibodies. (B) An independent experiment was also done up to day 4. Anti-TFIIB antibody was used for normalizing the amounts loaded. (C) Adipogenic stimulation enhances the repression activity of AEBP1. Confluent 3T3-L1 cells were transiently transfected with 2 μg of either paP2(-168)CAT (top panel) or pGALTKCAT (bottom panel) along with 1 μg of either pSVAEBP1(-) [A(-)] or pSVAEBP1 [A(+)]. After 1 and 2 days, cells were collected and analyzed for CAT activity. During 24 or 48 h incubation, the cells were treated with or without the adipogenic inducers (IDM) as indicated. The values are the means of three independent experiments, and were plotted as the mean ± SE ($n = 3$). Data are plotted relative to the activity for A(-). The significance of differences between A(+) and A(+)/IDM on days 1 and 2 was calculated as $*P < 0.002$ and $**P < 0.1$ by the Student's *t*-test.

stimulation, and found that their subcellular distributions were not changed significantly upon induction (data not shown). However, the abundance of Gγ5 was decreased selectively during the mitotic clonal expansion phase of adipocyte differentiation at days 1 and 2 (Figure 4A). Upon induction, post-confluent pre-adipocytes undergo several required rounds of mitotic clonal expansion prior to terminal differentiation (for a review, see Gregoire *et al.*, 1998). It was estimated that Gγ5 was decreased to ~10 and 50% of the initial level at days 1 and 2, respectively. Right after this period, the amount of Gγ5 returned to the day 0 level. No significant changes in the abundances of Gβ, Gγ7, ERKs, TFIIB and AEBP1 were observed over this period (Figure 4A and B; data not shown).

Adipogenic stimulation enhances the repression activity of AEBP1 at the onset of adipogenesis

The selective decrease in Gγ5 level during a specific stage of adipogenesis and its AEBP1-inhibitory activity suggest the possibility that these actions are linked directly and are physiologically relevant in adipogenesis. To test this hypothesis, we examined the transcriptional repression activity of AEBP1 upon adipogenic stimulation. The cells co-transfected with pSVAEBP1 along with paP2(-168)CAT showed ~35 and 70% of the CAT activity of cells transfected with the control plasmid pSVAEBP1(-) for 1 and 2 days, respectively (Figure 4C, top panel). When the cells transfected with pSVAEBP1 were treated with the adipogenic inducers (IDM), CAT activity was decreased further to ~20 and 50% of the CAT activity from cells

transfected with pSV(-)AEBP1 for 1 and 2 days, respectively (Figure 4C, top panel). This enhancement of repression activity upon adipogenic stimulation is specific for the *ap2* promoter, since no significant change in CAT activity was observed for cells transfected with a reporter plasmid (pGALTKCAT) containing a heterologous promoter (He *et al.*, 1995) (Figure 4C, low panel). The increased repression activity is likely to be due to the selective decrease in Gγ5 level mediated by the adipogenic stimulation (Figures 4A and B) because AEBP1 levels did not change during this period (data not shown). Finally, the adipogenic stimulation had no effect on the activity of another transcriptional repressor (AEBP2; He *et al.*, 1999) that does not interact with Gγ5 (data not shown). These results suggest that Gγ5 may be an important mediator that regulates AEBP1 transcriptional repression activity at the onset of adipogenesis.

Discussion

The regulation of a transcription factor by protein association is not unprecedented. A protein complex may prevent a transcription factor from binding to a specific DNA sequence or from interacting with basal or other transcription factors (for a review see Cowell, 1994). Apparently, the repression function of AEBP1 is regulated by preventing AEBP1 binding to DNA. Remarkably, the protein that prevents AEBP1 from repressing transcription is the γ5 subunit of a heterotrimeric G protein. This is the first report of a signal-transducing molecule being directly involved, by protein-protein interaction, in transcriptional

regulation during adipogenesis. In view of the tight association of the β and γ subunits *in vivo*, the inhibitory effect of G γ 5 may result from ternary complex formation between the G $\beta\gamma$ 5 dimer and AEBP1. However, it is conceivable that G γ 5 may also modulate the transcriptional repression function of AEBP1 directly, as seen in the *in vitro* experiments. It has been suggested that an intrinsic G γ monomer may exist in certain systems (Higgins and Casey, 1994; Morishita *et al.*, 1994; Herlitze *et al.*, 1996).

G γ 5 appears to function as a regulator for the transcriptional repression activity of AEBP1 during adipocyte differentiation. We speculate that these actions of G γ 5 and AEBP1 might provide a basis for the regulation of transcription at the onset of differentiation. In this regard, the selective decrease in G γ 5 level upon adipogenic stimulation seems to induce the DNA-binding and repression activity of AEBP1 during clonal expansion. We speculate that AEBP1 might regulate a certain gene(s) whose expression is altered transiently during clonal expansion. Recent studies suggest that growth-promoting transcriptional activities and a critical tyrosine dephosphorylation event are involved during mitotic clonal expansion at the onset of adipogenesis (Liao and Lane, 1995; Shugart *et al.*, 1995; Richon *et al.*, 1997). AEBP1 might also regulate the adipogenic gene encoding the transactivator C/EBP α . Expression of antisense AEBP1 RNA causes a marked induction of C/EBP α expression prior to adipocyte differentiation (S.-W.Kim and H.-S.Ro, unpublished). There are at least three C/EBP isoforms (α , β and δ) which are expressed during different stages of adipocyte differentiation. These proteins play vital roles in the initiation of adipogenesis and throughout the differentiation process. Typically, expression of C/EBP α occurs after the differentiation process has begun (for a review see Mandrup and Lane, 1997). Expression of C/EBP α at higher than physiological levels also induces adipogenesis in fibroblasts (Freytag *et al.*, 1994), whereas expression of antisense C/EBP α RNA suppresses adipogenesis in 3T3-L1 cells (Lin *et al.*, 1992). These results have suggested that C/EBP α is a key regulator and plays a vital role in a mechanism which results in adipogenesis. The transcriptional factor AP-2 α /CUP (C/EBP α -undifferentiated protein) was shown to be involved in repressing the C/EBP α gene in pre-adipocytes and, upon induction of differentiation, AP-2 α /CUP expression is down-regulated, allowing expression of C/EBP α (Vasseur-Cognet and Lane, 1993; Tang *et al.*, 1997; Jiang *et al.*, 1998). AP-2 α /CUP expression, which persists during clonal expansion, is abolished at day 4 post-induction when the expression of the C/EBP α gene is induced. Since C/EBP α has been shown to induce a set of genes involved in growth arrest (Constance *et al.*, 1996), which is required for establishing the differentiation stage, repression of the C/EBP α gene during clonal expansion appears to be crucial. AEBP1 may be involved in the repression of the C/EBP α gene. The enhanced repression activity of AEBP1 during clonal expansion would ensure tight repression of the C/EBP α gene during this phase. Determination of the mechanism of repression of the C/EBP α gene by AEBP1 and assessment of its effects on the differentiation program are important future studies.

Materials and methods

Materials

Protein A-agarose and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse or anti-rabbit antibodies were purchased from Santa Cruz Biotechnology, as were polyclonal antibodies directed against G γ 5, G γ 7, G β , PKA and IGFR. Monoclonal antibody directed against the HA epitope was purchased from Boehringer Mannheim. Dulbecco's modified Eagle's medium (DMEM) and bovine calf serum were purchased from Gibco-BRL. The cosmic calf serum was purchased from HyClone Laboratories. The HeLa nuclear extract was purchased from Promega.

Yeast two-hybrid screening

Yeast strain Y153 harboring pGBT-AEBP1, an expression plasmid encoding full-length AEBP1 fused to the Gal4 DNA-binding domain, was transformed with a human cDNA library that was constructed in the vector pACT. A positive clone (pACT-G γ 5) encoding the γ 5 subunit of a heterotrimeric G protein fused to the Gal4 activation domain was obtained from $\sim 7 \times 10^6$ transformants. Interaction between fusion proteins expressed by the specific plasmids was indicated by the growth of yeast transformants on selective medium (SC-Leu, Trp and His) containing 60 mM 3-aminotriazole. His⁺ colonies were analyzed for β -galactosidase activity using a filter lift procedure (Durfée *et al.*, 1993).

Immunoprecipitation

3T3-L1 cells were collected in cold RIPA buffer containing 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μ g aprotinin/ml, 10 μ g leupeptin/ml, 5 mM EDTA and 5 mM EGTA. The cell lysates were incubated with protein A-agarose for 1 h, then the beads were discarded and the supernatants were incubated with specific antibodies (1 μ g/ml anti-G γ 5 or anti-G β , 2 μ g/ml anti-HA, anti-PKA or anti-IGFR, or 2.5 μ g/ml of either affinity-purified anti-AEBP1 antibodies or IgG from normal serum) for 1 h and then overnight with protein A-agarose. Samples were collected and washed four times with RIPA buffer. The precipitated samples were resolved by SDS-PAGE and analyzed by Amersham's ECL blotting system.

Subcellular fractionation

Fractionation was performed on ice or at 4°C as described (Oláh *et al.*, 1994) with modifications. Cells were collected into 500 μ l of fractionation buffer (2 mM EDTA, 2 mM EGTA, 20 mM Tris-HCl pH 7.5) supplemented with 25 μ g leupeptin/ml, 25 μ g aprotinin/ml and 1 mM PMSF, and then disrupted by 25 passages through a needle (25 gauge) on ice. Cells were centrifuged for 5 min at 1000 g, and the pellet, which represents the nuclear fraction (N), was washed and centrifuged twice with fractionation buffer. The nuclear fraction was examined under a light microscope and found to contain intact nuclei without any lysed cells. The supernatant was centrifuged for 1 h at 19 000 g to separate the plasma membrane-enriched fraction (PP) and soluble cytosolic fraction (S). Equal amounts of these fractions were mixed with sample buffer and resolved by 8.5 or 15% SDS-PAGE, then transferred to nitrocellulose membrane on ice or at 70°C (G γ analysis; Robishaw and Balcueva, 1993) and analyzed by Amersham's ECL or ECL-plus blotting system. To assess the degree of contamination by cytosolic proteins of the N fraction, a known cytosolic protein, acetyl-CoA carboxylase (AC), was used as a marker protein. Since AC is biotinylated in cells (Geelan *et al.*, 1997), the immunoblotting was carried out with horseradish peroxidase (HRP)-conjugated avidin (Haneji *et al.*, 1993). As nuclear markers, the c-Myc transcription factor and the general transcription factor TFIIB, that binds to DNA along with TFIID near the TATA box of promoters, were used. IGFR, a transmembrane protein, was used as a plasma membrane marker.

Immunofluorescent confocal laser microscopy

Cells were grown on 18 $\times$ 18 mm No. 1 coverslips, washed twice with phosphate-buffered saline (PBS) and fixed with 4% formaldehyde in PBS for 10 min at room temperature. Cells were permeabilized with 0.01% saponin (Sigma) in PBS for preservation of G protein subunits in the plasma membrane (Hansen *et al.*, 1994), washed with PBS then blocked by incubation with 5% fat-free milk powder in PBS for 30 min at room temperature. Samples were incubated with primary antibodies (2 μ g/ml for G β and G γ 5, 4 μ g/ml for G γ 7 and AEBP1) overnight at 4°C and with FITC-conjugated secondary antibody (3.5 μ g/ml) for 1 h at room temperature. Samples were examined with confocal laser microscopy (Carl Zeiss) to photograph the nuclear sections at 3 μ m from the bottom of the cells.

Cell culture and differentiation

3T3-L1 cells were cultured in DMEM containing 10% bovine calf serum until confluent, referred to as day 0, then switched to differentiation medium containing 10% cosmic calf serum, 10 µg of insulin/ml, 1 µM dexamethasone and 0.5 mM 1-methyl-3-isobutylxanthine for 2 days (Green and Kehinde, 1975; Russell and Ho, 1976). Differentiating cells were maintained in DMEM containing 10% cosmic calf serum and 10 µg of insulin/ml with a change of medium at an interval of 3 days.

Transfection and CAT assay

3T3-L1, NIH 3T3 and COS-7 cells were cultured in 90 mm dishes and transfected at 80% confluence by the polybrene procedure as described (He *et al.*, 1995). Detailed descriptions of plasmid constructions (pJ3H-AEBP1, pRc/CMVγ5 and pCMV/AE-1) are available from the authors on request. Transfection was performed with 2.5 µg of the reporter plasmid pAP2(-168)CAT and 5 µg of the AEBP1 expression plasmid pSVAEBP1 [or the control plasmid pSVAEBP1(-)] along with various amounts of the expression plasmid encoding the γ5 subunit (pRc/CMVγ5). The γ5 cDNA was also cloned into expression vector pRc/CMV (Invitrogen) in the opposite orientation [pRc/CMVγ5(-)] to serve as a negative control. All transfections also included 1 µg of pHermes-lacZ. β-galactosidase activity was assayed 48 h after transfection to normalize transfection efficiency, and CAT activity was assayed as described (He *et al.*, 1995).

In vitro transcription assay

Nuclear run-off experiments were carried out using HeLa nuclear extract according to the manufacturer's instructions with pCMV or pCMV/AE-1 templates (see Figure 3B). Histidine-tagged recombinant AEBP1 protein (He *et al.* 1995) (200 ng) was pre-incubated with various amounts of histidine-tagged recombinant γ5 protein in a buffer containing 1× HeLa nuclear extract buffer [20 mM HEPES (pH 7.9), 100 mM KCl, 0.2 mM EDTA, 0.5 mM dithiothreitol (DTT), 20% glycerol], 3 mM MgCl₂, 0.4 mM each of ATP, CTP and UTP, 0.016 mM GTP, 40 U of RNase inhibitor, 10 µCi of [α-³²P]GTP and 10 ng of template DNA for 30 min on ice; then 8 U of HeLa nuclear extract was added to start transcription (25 µl in total volume) and incubation was continued at 30°C for 1 h. The reaction was stopped by adding 175 µl of stop mixture (300 mM Tris-HCl pH 7.4, 300 mM NaOAc, 0.5% SDS, 2 mM EDTA, 3 µg of tRNA/ml). The product was resolved by electrophoresis on a 6% denaturing polyacrylamide gel and exposed to X-ray film overnight at -70°C with an intensifying screen.

EMSA for AEBP1-Gy5 interaction

Recombinant AEBP1 protein was pre-incubated with recombinant γ5 or control (recombinant Rab11) proteins for 10 min at room temperature in binding buffer (100 mM Tris pH 7.5, 100 mM KCl, 50 mM MgCl₂, 10 mM DTT and 25% glycerol). ³²P-Labeled AE-1 oligonucleotides were added and incubated for a further 20 min at room temperature. For competition studies (Figure 3E, lane 8), an ~200-fold molar excess of cold unlabeled AE-1 oligonucleotides was added to the binding reaction.

Acknowledgements

We thank R.A.Singer and N.D.Ridgway for discussion and comments on the manuscript; S.J.Elledge for the human library in pACT vector; J.Chernoff for plasmid pJ3H; P.P.Poon for the recombinant Rab11 protein; all the members of the Yeast Group for help in the yeast two-hybrid screening; J.Shi for technical assistance; and the Department of Biology for immunofluorescent confocal laser microscopy. This work was supported by grants from the Heart and Stroke Foundation (Nova Scotia) of Canada, the Canadian Diabetes Association and NSERC to H.-S.R. We acknowledge the support of a Walter C.Sumner Memorial Fellowship to A.M. and an HSFC Research Scholarship to H.-S.R.

References

Bégin-Heick,N., Black,M.A., Gaffield,J. and Cadrin,M. (1997) Subcellular localization of G-proteins in primary-cultured mouse preadipocytes and adipocytes. *J. Cell. Biochem.*, **65**, 259–266.
 Boyartchuk,V.L., Ashby,M.N. and Rine,J. (1997) Modulation of Ras and a-factor function by carboxyl-terminal proteolysis. *Science*, **275**, 1796–1800.
 Cao,Z., Umek,R.M. and McKnight,S.L. (1991) Regulated expression of three C/EBP isoforms during adipose conversion of 3T3 L1 cells. *Genes Dev.*, **5**, 1538–1552.

Chien,C.-T., Bartel,P.L., Sternglanz,R. and Fields,S. (1991) The two-hybrid system: a method to identify and clone genes for proteins that interact with a protein of interest. *Proc. Natl Acad. Sci. USA*, **88**, 9578–9582.
 Christy,R.J., Yang,V.W., Ntambi,J.M., Geiman,D.E., Landschulz,W.H., Friedman,A.D., Nakabeppu,Y., Kelly,T. and Lane,M.D. (1989) Differentiation-induced gene expression in 3T3 L1 preadipocytes: CCAAT/enhancer binding protein interacts with and activates the promoters of two adipocyte-specific genes. *Genes Dev.*, **3**, 1323–1335.
 Clapham,D.E. and Neer,E.J. (1997) G protein βγ subunits. *Annu. Rev. Pharmacol. Toxicol.*, **37**, 167–203.
 Constance,C.M., Morgan,J.I. and Umek,R.M. (1996) C/EBPα regulation of the growth-arrest-associated gene *gadd45*. *Mol. Cell. Biol.*, **16**, 3878–3883.
 Cook,L.A., Schey,K.L., Wilcox,M.D., Dingus,J. and Hildebrandt,J.D. (1998) Heterogeneous processing of a G protein γ subunit at a site critical for protein and membrane interactions. *Biochemistry*, **37**, 12280–12286.
 Cowell,I.G. (1994) Repression versus activation in the control of gene transcription. *Trends Biochem. Sci.*, **19**, 38–42.
 Crouch,M.F. (1991) Growth factor-induced cell division is paralysed by translocation of Giα to the nucleus. *FASEB J.*, **5**, 200–206.
 Crouch,M.F. and Simson,L. (1997) The G-protein Gi regulates mitosis but not DNA synthesis in growth factor-activated fibroblasts: a role for the nuclear translocation of Gi. *FASEB J.*, **11**, 189–198.
 Distel,R.J., Ro,H.-S., Rosen,B.S., Groves,D.L. and Spiegelman,B.M. (1987) Nucleoprotein complexes that regulate gene expression in adipocyte differentiation: direct participation of c-fos. *Cell*, **49**, 835–844.
 Durfee,T., Becherer,K., Chen,P.L., Yeh,Y., Kilburn,A.E., Lee,W.H. and Elledge,S.J. (1993) The retinoblastoma protein associates with the protein phosphatase type 1 catalytic subunit. *Genes Dev.*, **7**, 555–569.
 Fisher,K.J. and Aronson,N.N., Jr (1992) Characterization of the cDNA and genomic sequence of a G protein γ subunit (γ5). *Mol. Cell. Biol.*, **12**, 1585–1591.
 Freytag,S.O., Paielli,D.L. and Gilbert,J.D. (1994) Ectopic expression of CCAAT/enhancer-binding protein α promotes adipogenic program in a variety of mouse fibroblastic cells. *Genes Dev.*, **8**, 1654–1663.
 Geelan,M.J.H., Bijleveld,C., Velasco,G., Wanders,R.J.A. and Guzman,M. (1997) Studies on the intracellular localization of acetyl-CoA carboxylase. *Biochem. Biophys. Res. Commun.*, **233**, 253–257.
 Green,H. and Kehinde,O. (1975) An established preadipose cell line and its differentiation in culture. II. Factors affecting the adipose conversion. *Cell*, **5**, 19–27.
 Gregoire,F.M., Smas,C.M. and Sul,H.S. (1998) Understanding adipocyte differentiation. *Physiol. Rev.*, **78**, 783–809.
 Haneji,T., Toyama,Y. and Nagano,T. (1993) Detection of endogenous avidin-binding proteins in rat liver cells by transblot and electron microscopy. *J. Electron Microsc.*, **42**, 231–235.
 Hansen,C.A., Schroering,A.G., Carey,D.J. and Robishaw,J.D. (1994) Localization of a heterotrimeric G protein γ subunit to focal adhesions and associated stress fibers. *J. Cell Biol.*, **126**, 811–819.
 Harper,J.W., Adami,G.R., Wei,N., Keyomarsi,K. and Elledge,S.J. (1993) The p27 Cdk-interacting protein Cip1 is a potent inhibitor of G₁ cyclin-dependent kinases. *Cell*, **75**, 805–816.
 He,G.-P., Muise,A., Wu Li,A. and Ro,H.-S. (1995) A eukaryotic transcriptional repressor with carboxypeptidase activity. *Nature*, **378**, 92–96.
 He,G.-P., Kim,S.-W. and Ro,H.-S. (1999) Cloning and characterization of a novel zinc-finger transcriptional repressor: a direct role of the zinc-finger motif in repression. *J. Biol. Chem.*, **274**, 14678–14684.
 Herlitz,S., Garcia,D.E., Mackie,K., Hille,B., Scheuer,T. and Catterall,W.A. (1996) Modulation of Ca²⁺ channels by G-protein βγ subunits. *Nature*, **380**, 258–262.
 Herrera,R., Ro,H.-S., Robinson,G.S., Xanthopoulos,K.G. and Spiegelman,B.M. (1989) A direct role for C/EBP and the AP-1-binding site in gene expression linked to adipocyte differentiation. *Mol. Cell. Biol.*, **9**, 5331–5339.
 Higgins,J.B. and Casey,P.J. (1994) *In vitro* processing of recombinant G protein γ subunits. *J. Biol. Chem.*, **269**, 9067–9073.
 Hunt,C., Ro,H.-S., Min,H.-Y., Dobson,D.E. and Spiegelman,B.M. (1986) Adipocyte P2 gene: developmental expression and homology of 5'-flanking sequences among fat cell-specific genes. *Proc. Natl Acad. Sci. USA*, **83**, 3786–3790.

- Jiang,M., Tang,Q., McLenithan,J., Geiman,D., Shillinglaw,W., Henzel,W.J. and Lane,M.D. (1998) Derepression of the C/EBP α gene during adipogenesis: identification of AP-2 α as a repressor. *Proc. Natl Acad. Sci. USA*, **95**, 3467–3471.
- Liao,K. and Lane,M.D. (1995) The blockade of preadipocyte differentiation by protein-tyrosine phosphatase HA2 is reversed by vanadate. *J. Biol. Chem.*, **270**, 12123–12132.
- Lin,F.T. and Lane,M.D. (1992) Antisense CCAAT/enhancer-binding protein RNA suppresses coordinate gene expression and triglyceride accumulation during differentiation of 3T3-L1 preadipocytes. *Genes Dev.*, **6**, 533–544.
- Mandrup,S. and Lane,M.D. (1997) Regulating adipogenesis. *J. Biol. Chem.*, **272**, 5367–5370.
- Matarese,V. and Bernlohr,D.A. (1988) Purification of murine adipocyte lipid-binding protein. Characterization as a fatty acid- and retinoic acid-binding protein. *J. Biol. Chem.*, **263**, 14544–14551.
- Morishita,R., Kato,K. and Asano,T. (1994) A brain-specific γ subunit of G protein freed from the corresponding β subunit under non-denaturing conditions. *FEBS Lett.*, **337**, 23–26.
- Oláh,Z., Lehel,C., Anderson,W.B., Brenneman,D.E. and van Agoston,D. (1994) Subnanomolar concentration of VIP induces the nuclear translocation of protein kinase C in neonatal rat cortical astrocytes. *J. Neurosci. Res.*, **39**, 355–363.
- Richon,V.M., Lyle,R.E. and McGehee,R.E.,Jr (1997) Regulation and expression of retinoblastoma proteins p107 and p130 during 3T3-L1 adipocyte differentiation. *J. Biol. Chem.*, **272**, 10117–10124.
- Ro,H.-S. and Roncari,D.A.K. (1991) The C/EBP-binding region and adjacent sites regulate expression of the adipose P2 gene in human preadipocytes. *Mol. Cell. Biol.*, **11**, 2303–2306.
- Robishaw,J.D. and Balcueva,E.A. (1993) A high temperature transfer procedure for detection of G protein γ subunits by immunoblotting. *Anal. Biochem.*, **208**, 283–287.
- Russell,T. R. and Ho,R. (1976) Conversion of 3T3 fibroblasts into adipose cells: triggering of differentiation by prostaglandin F $_{2\alpha}$ and 1-methyl-3-isobutylxanthine. *Proc. Natl Acad. Sci. USA*, **73**, 4516–4520.
- Shugart,E.C., Levenson,A.S., Constance,C.M. and Umek,R.M. (1995) Differential expression of gas and gadd genes at distinct growth arrest points during adipocyte development. *Cell Growth Differ.*, **6**, 1541–1547.
- Su,H.-L., Malbon,C.C. and Wang,H.-Y. (1993) Increased expression of G α 2 in mouse embryo stem cells promotes terminal differentiation to adipocytes. *Am. J. Physiol.*, **265**, C1729–C1735.
- Tang,Q., Jiang,M. and Lane,M.D. (1997) Repression of transcription mediated by dual elements in the CCAAT/enhancer binding protein α gene. *Proc. Natl Acad. Sci. USA*, **94**, 13571–13575.
- Ueda,H., Saga,S., Shinohara,H., Morishita,R., Kato,K. and Asano,T. (1997) Association of the γ 12 subunit of G proteins with actin filaments. *J. Cell Sci.*, **110**, 1503–1511.
- Vasseur-Cognet,M. and Lane,M.D. (1993) CCAAT/enhancer binding protein α (C/EBP α) undifferentiated protein: a developmentally regulated nuclear protein that binds to the C/EBP α gene promoter. *Proc. Natl Acad. Sci. USA*, **90**, 7312–7316.
- Wang,H.-Y., Watkins,D.D. and Malbon,C.D. (1992) Antisense oligodeoxynucleotides to Gs protein subunit sequence accelerate differentiation of fibroblasts to adipocytes. *Nature*, **358**, 334–337.
- Zhang,F.L. and Casey,P.J. (1996) Protein prenylation: molecular mechanisms and functional consequences. *Annu. Rev. Biochem.*, **65**, 241–269.

Received April 12, 1999; revised and accepted May 24, 1999