Effect of heterologous expression of acyl-CoA-binding protein on acyl-CoA level and composition in yeast

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We have expressed a bovine synthetic acyl-CoA-binding protein (ACBP) gene in yeast (*Saccharomyces cerevisiae*) under the control of the *GAL1* promoter. The heterologously expressed bovine ACBP constituted up to 6.4% of total cellular protein and the processing was identical with that of native bovine ACBP, i.e. the initiating methionine was removed and the following serine residue was *N*-acetylated. The expression of this protein did not affect the growth rate of the cells. Determination of the yeast acyl-CoA pool size showed a close positive correlation between the ACBP content of the cells and the size of the acyl-CoA pool. Thus ACBP can act as an intracellular acyl-CoA pool former. Possible physiological functions of ACBP in cells are discussed.

INTRODUCTION

Acyl-CoA-binding protein (ACBP) was first described by Mogensen et al. (1987). The protein was identified by its ability to bind acyl-CoA and to induce medium-chain acyl-CoA synthesis by goat mammary-gland fatty acid synthetase. When ACBP from bovine and rat liver was sequenced (Mikkelsen et al., 1987; Knudsen et al., 1989), it turned out to be identical with diazepam-binding inhibitor (Knudsen et al., 1989). Diazepam-binding inhibitor was first isolated from rat brain and identified by its ability to displace diazepam from the benzodiazepine-binding site on the γ-aminobutyric acid receptor complex ($GABA_A$) (Guidotti et al., 1983). The same protein was later isolated from bovine brain and given the name endozepine (Shoyab et al., 1986). These groups conclude that ACBP (diazepam-binding inhibitor) is a natural modulator of GABA stimulation of the $GABA_A$ complex (Costa and Guidotti, 1991). Recent studies have indicated that this protein might also be involved in steroid-hormone synthesis (Besman et al., 1989; Papadopoulos et al., 1991) and regulation of glucose-induced insulin secretion (Östenson et al., 1990).

The function of ACBP as a modulator of the $GABA_A$ receptor complex has been questioned (Knudsen and Nielsen, 1990). The main arguments against ACBP being a natural modulator of GABA stimulation are that, in the original work (Costa et al., 1983), large concentrations (in the micromolar range) were needed to displace benzodiazepines from $GABA_A$, and that these results could not be repeated with ACBP purified from bovine liver (Knudsen and Nielsen, 1990). Direct binding of ACBP to the $GABA_A$ receptor has never been shown. Furthermore, ACBP is present in all tissues tested, with the highest concentration being found in the liver (Mikkelsen et al., 1987; Knudsen et al., 1989; Bovolin et al., 1990), and finally recent results obtained by *in vitro* hybridization indicate that ACBP is only expressed at a very low level in neurons (Tong et al., 1991).

A role of ACBP in the acute regulation of steroid-hormone secretion, as previously suggested by Yanagibashi et al. (1988) and Besman et al. (1989), is very unlikely as it has recently been shown that ACBP synthesis and concentration in isolated steroid-producing cells is not affected by adrenocorticotropin or luteinizing hormone (Brown et al., 1992). Furthermore, the increase in plasma corticosterone level after adrenocorticotropin replacement in hypophysectomized rats precedes the increase in ACBP in the adrenal gland (Massotti et al., 1991).

There are several indications that ACBP functions as an intracellular acyl-CoA transporter and acyl-CoA pool former. ACBP binds acyl-CoA esters with high affinity *in vitro* (Mikkelsen et al., 1987; Knudsen et al., 1989), it can compete with other cell proteins and membranes in binding acyl-CoA *in vitro* (Rasmussen et al., 1990) and it has been possible to bind and photocross-link a photoreactive radioactive acyl-CoA derivative to ACBP (Hach et al., 1990).

If ACBP functions as an intracellular acyl-CoA transporter and acyl-CoA-pool former, it can be predicted that over-expression of ACBP in a given cell would result in an increased intracellular pool size of acyl-CoA. To test this hypothesis, a bovine synthetic ACBP gene was expressed in yeast (*Saccharomyces cerevisiae*). The results from these *in vivo* experiments show that a high-level expression of ACBP has the expected effect on the acyl-CoA level, supporting the hypothesis that ACBP is an intracellular acyl-CoA transporter and pool former.

MATERIALS AND METHODS

Materials

Yeast nitrogen base (without amino acids) was from Difco Laboratories, Detroit, MI, U.S.A. Q-Sepharose fastflow and Sephadex G-50 superfine were obtained from Pharmacia Biosystem, Uppsala, Sweden. Nucleosil ODS (10 μm particle size, 100 nm pore size) and Nucleosil ODS (10 μm particle size, 300 nm pore size) were from Machery-Nagel, Düren, Germany. *Staphylococcus aureus* U8 proteinase (SAP; EC 3.4.21.19) was from Miles Laboratories and trypsin (EC 3.4.21.4) from Boehringer, Mannheim. Propan-2-ol (h.p.l.c. grade) and trifluoroacetic acid (gas-phase sequenator grade) were from Rathburn, Walkerburn, Scotland, U.K.

Strains of *S. cerevisiae* and *Escherichia coli* and plasmid

The yeast strain JHRY 20-2C-del-2 (*MAT a, his3-del-200, leu 2-*

Abbreviations used: ACBP, acyl-CoA-binding protein; GABA, γ-aminobutyric acid; SAP, *Staphylococcus aureus* proteinase.

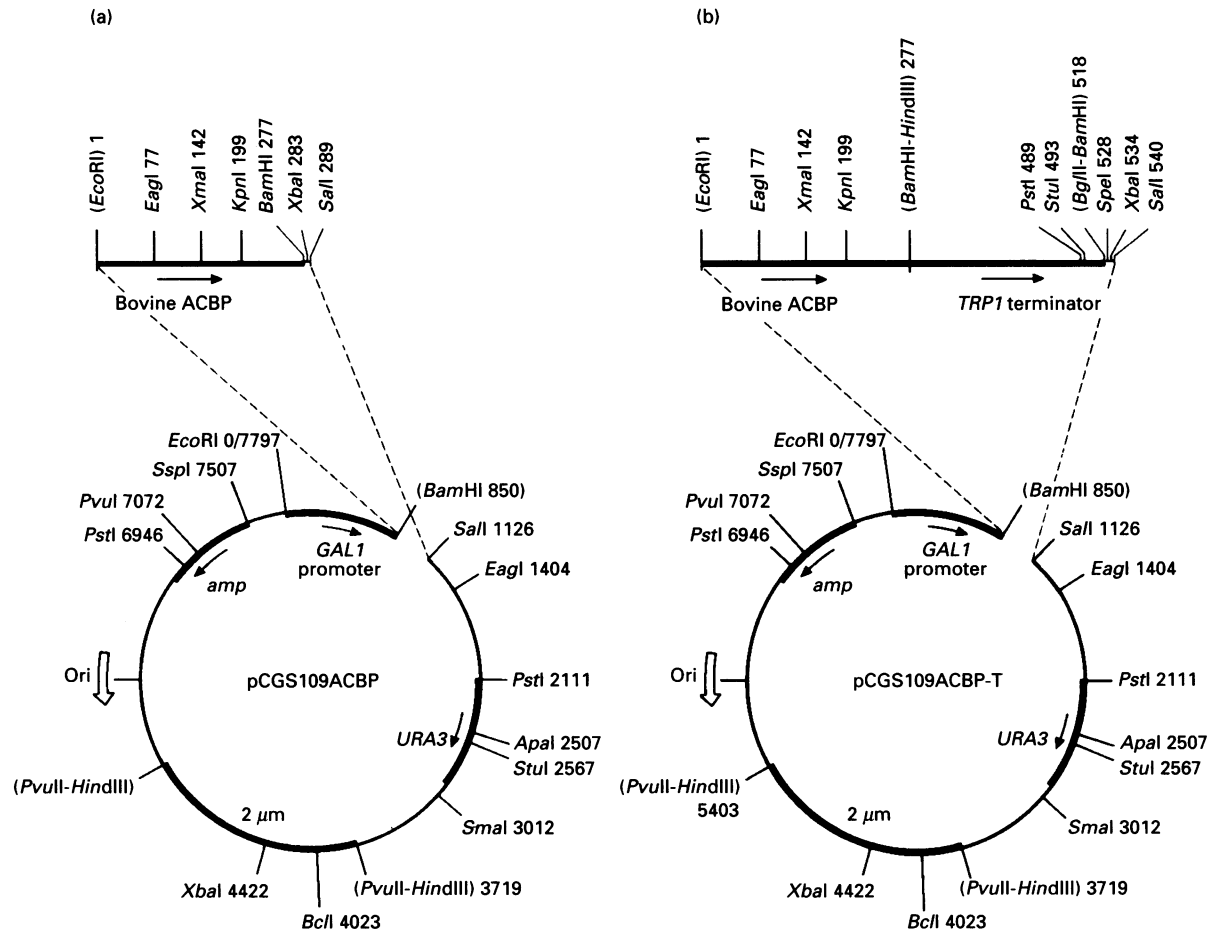

Figure 1 The pCGS109ACBP (a) and pCGS109ACBP-T (b) vectors used for expression of bovine ACBP in yeast

The numbering of the cloning vector and the insert refer to the number of nucleotides from the *Eco*RI site in the pCGS109 vector (clockwise rotation) and the *Eco*RI site in the ACBP insert respectively.

3, leu 2-112, ura3-52, pep4-del-2::LEU 2) was kindly supplied by Morten Kielland Brandt, Carlsberg Laboratory, Copenhagen, Denmark. The pCGS109 vector (Botstein et al., 1987) was obtained from Donald T. Moir, Collaborative Research Inc. To make pCGS109, a *Bam*HI linker (CCCGGATCCGG) had been ligated to the *GAL10–GAL1* promoter (Johnston and Davis, 1984) at nucleotide 819, and the *Eco*RI–*Bam*HI fragment containing the entire promoter was inserted between the *Eco*RI and *Bam*HI sites of pCGS40 (Goff et al., 1984).

The *E. coli* strain used for propagation of plasmids was DH5α (*F⁻, recA1, endA1, gyrA96, thi, hsdR17* (r_K^-, m_K^+), *sup E44, relA1, λ-Φ 80 dlac 2 Δ M15*).

Growth media

Growth media for yeast transformed with the expression plasmids and used for production of recombinant bovine ACBP were composed as follows: 5 litres of medium contained 50 g of succinate, 30 g of NaOH, 10 g of $(NH_4)_2HPO_4$, 1.25 g of K_2HPO_4, 8.75 g of KH_2PO_4, 5 g of $MgSO_4$, 7 H_2O, 1 g of NaCl, 1.05 g of $CaCl_2$, 200 mg of arginine, histidine and adenine sulphate, 300 mg of valine, tryptophan, leucine, isoleucine, lysine and tyrosine, 500 mg of phenylalanine, 600 mg of methionine, 1 g of glutamic acid, 37.5 g serine, 3 g of aspartic acid, 6 g of threonine, 200 g of galactose, 100 μl of each of the solutions 0.1% H_3BO_3, 0.1% $CuSO_4$, 0.1% KI, 0.5% $FeCl_3$ and 0.7% $ZnSO_4$ and 10 ml of vitamin stock solution containing 0.2 mg of biotin, 40 mg of thiamine, pyridoxine and calcium pantothenate and 200 mg of inositol/100 ml.

Growth media for cultures used for determination of growth curves, acyl-CoA and ACBP content contained 6.7 g/l yeast nitrogen base (without amino acids), 20 g/l galactose, 20 mg/l histidine and 30 mg/l leucine. Cultures (500 ml) were grown with shaking in 5-litre conical flasks at 30 °C.

Construction of pCGS109ACBP and pCGS109ACBP-T

To make pCGS109ACBP (Figure 1a), the synthetic bovine ACBP gene (Mandrup et al., 1991) was excised as a 288 bp *Eco*RI–*Sal*I fragment from pUC19ACBP and cloned between the *Bam*HI and *Sal*I sites of pCGS109.

The plasmid pCGS109ACBP-T (Figure 1b) contains the yeast *TRP1* terminator inserted immediately 3′ of the ACBP gene. To make this construct, the terminator (Zaret and Sherman, 1982) was excised from the *TRP1* gene as a *Hin*dIII–*Bgl*II fragment (nucleotide 615–852; Tschumper and Carbon, 1980) and inserted in pBluescript KS(+) (Stratagene). The *Hin*dIII–*Xba*I fragment containing the *TRP1* terminator was excised and inserted between the *Bam*HI and *Xba*I sites in pUC19ACBP to make pUC19ACBP-T. Subsequently, the *Eco*RI–*Sal*I fragment of this

plasmid containing the ACBP gene and the *TRP1* terminator was cloned between the *Bam*HI and *Sal*I sites of pCGS109.

All plasmid constructions were propagated in the *E. coli* strain DH5α. The yeast strain JHRY 20-2C-del-2 was then transformed with the two expression plasmids as described by Ito et al. (1983).

Purification of recombinant bovine ACBP

Cells grown to a density of $A_{550} = 17.5$ in a 5-litre fermenter with automatic pH control were harvested by centrifugation at 3000 *g* for 30 min. The pelleted cells were washed with 0.5 vol. of 0.9% (w/v) NaCl and repelleted.

The repelleted cells were resuspended in 1 M acetic acid using a volume equal to that of the cells and passed twice through a French press at a pressure of 3450 kPa. The resulting pH was 3.5–4.0. The pH was then adjusted to 7.0 with 5 M NaOH and the solution cleared by centrifugation at 10^4 *g* for 20 min. The pellet was discarded and the supernatant was heated at 50 °C for 20 min, placed on ice and cleared by centrifugation at 10^4 *g* for 20 min. The supernatant was then loaded on to a Sephadex G-50 superfine column (5 cm × 80 cm) equilibrated with 30 mM Tris/HCl, pH 9.0, 0.02% (w/v) sodium azide. The proteins were eluted with the same buffer with a flow of 80 ml/h. The ACBP-containing fractions were loaded on a Q Sepharose fast-flow ion-exchange column (2.5 cm × 32 cm) equilibrated with 30 mM Tris/HCl, pH 9.0 (buffer A). The proteins were eluted with a gradient of 0.5 M NaCl in 30 mM Tris/HCl, pH 9.0 (buffer B). The gradient of buffer B in A was as follows: 0–10% B in 10 min, 10–20% B in 40 min, 20–35% B in 80 min.

The ACBP-containing fraction from the ion-exchange column was pooled and further purified by reversed-phase h.p.l.c. on a Nucleosil ODS 10/100 C_{18} column (8 mm × 120 mm) equilibrated with 20% solution B [50% (v/v) propan-2-ol, 0.1% (v/v) trifluoroacetic acid in water] in solution A [0.1% (v/v) trifluoroacetic acid in water]. ACBP was eluted with a gradient of solution B in solution A as follows; 20% B to 80% B in 42 min with a flow of 3 ml/min. ACBP-containing fractions were pooled and freeze-dried.

Characterization of recombinant ACBP

The structure of ACBP was verified by SAP and trypsin digestion followed by analysis by plasma desorption m.s. as previously described (Mikkelsen et al., 1987).

Protein determination

Total protein was determinated by a modified Lowry method described by Schachterle and Pollack (1973). ACBP concentrations in solutions of pure ACBP were determined by u.v. absorption using $A^{1\%}_{1cm,280} = 15.5$.

Electrophoresis

SDS/PAGE was carried out on 20% (w/v) homogeneous gels in the Pharmacia Phast System using the conditions recommended by the manufacturer.

Determination of bovine ACBP content in yeast by e.l.i.s.a.

Yeast cells (2×10^8) were harvested by centrifugation at 3000 *g* for 10 min and washed with 1 ml of 0.9% (w/v) KCl. The cells were resuspended in 600 μl of 1.0 mM phenylmethanesulphonyl fluoride, and 600 μl of dry glass pearls (0.25–0.3 mm) was added. The suspension was vortexed (6 × 30 s with intermittent cooling on ice). The supernatant was transferred to an Eppendorf tube and cleared by centrifugation at 18000 *g* for 1 min. Samples were taken for protein and ACBP determination and stored at −80 °C until analysis could be performed. It was routinely checked that more than 90% of the cells were broken by this procedure.

Determinations of total soluble protein and ACBP by e.l.i.s.a. were as described previously (Hansen et al., 1991), except that rabbit anti-(bovine ACBP) and biotinylated rabbit anti-(bovine ACBP) antibodies were used.

Extraction and reversed-phase h.p.l.c. of acyl-CoA esters

Yeast cells (approx. 5×10^9) were harvested by centrifugation at 3000 *g* for 15 min and resuspended in water to a final volume of 0.8 ml. The suspension was transferred to a −80 °C precooled Eton Press and extruded through a 1 mm hole by applying a high pressure on the piston. The frozen extruded yeast suspension was quantitatively transferred to a precooled 25 ml glass centrifuge tube. Acyl-CoA esters were extracted and analysed by reversed-phase h.p.l.c. as described by Rosendal and Knudsen (1992). Heptadecanoyl-CoA was added as internal standard. Chloroform/methanol (2:1, v/v) (8 ml) was added. Water was added to give a final chloroform/methanol/water ratio of 2.5:1.3:1.0. After thorough mixing using an Ultra-Turrax homogenizer, the two phases were separated by centrifugation at 3200 *g* for 45 min. The upper phase was transferred to a new tube. The interphase protein layer was loosened and the chloroform phase decanted and discarded. The protein layer was extracted three times with 2.0 M ammonium acetate, pH 6.9/methanol (1:4, v/v). The extracts were combined with the upper phase and taken to dryness in a vacuum concentrator. The dry residue was re-extracted with 0.7 ml of 25 mM potassium phosphate buffer, pH 5.3, containing 1.0 mg of ACBP/ml. The overall recovery of added [1-^{14}C]octadecanoyl-CoA was about 60%.

The extracted acyl-CoAs were analysed on a nucleosil ODS 10/100 column (4.6 mm × 250 mm). The mobile phases were, A, 20% acetonitrile in 25 mM KH_2PO_4, pH 5.3, and, B, 80% acetonitrile in 25 mM KH_2PO_4, pH 5.3. The column was equilibrated with 15% B in A. The gradients for eluting the acyl-CoA esters were as follows: 15% B for 15 min, 15% B to 25% B in 5 min, 25% B for 15 min, 25% B to 50% B in 55 min, 50% B for 5 min, 50% B to 85% B in 5 min, 85% B for 5 min, and the flow was 1 ml/min. The column was equilibrated for 20 min between each run. Samples were injected automatically using a WISP 710A autosampler. The acyl-CoA esters were detected by measuring u.v. absorption at 254 nm.

RESULTS AND DISCUSSIONS

Construction of expression plasmids

A synthetic gene encoding the 86 amino acid residues of mature bovine ACBP and the initiating methionine residue was constructed and expressed in *E. coli* (Mandrup et al., 1991). The codon usage and the sequences flanking the coding region were designed in such a way that transcripts from the gene would be efficiently translated not only in *E. coli* but also in *S. cerevisiae*. Thus, this synthetic gene was transferred to the yeast expression vector pCGS109. Two constructs were made, pCGS109ACBP and pCGS109ACBP-T (Figure 1). In both of these, transcription of the ACBP gene is driven by the galactose-inducible *GAL1* promoter giving rise to transcripts with a 5′-leader of approx. 60 nt which contains no alternative AUG start codons. In pCGS109ACBP, the synthetic ACBP gene was inserted between

Table 1 M_r determinations of SAP and trypsin fragments of recombinant bovine ACBP expressed in yeast

The purified recombinant bovine ACBP was digested with SAP and trypsin, the peptides were separated by h.p.l.c. and the M_r determined by plasma desorption m.s. See the Materials and methods section for further details.

Fraction	M_r Observed	M_r Expected	Deviation (%)
S1	—	478.0	—
S2	809.8	808.8	0.12
S3	1395.2	1394.6	0.04
S4	4368.0	4361.9	0.14
S5	762.7	761.9	0.11
S6	1411.9	1411.6	0.02
S7	849.6	849.1	0.06
T1	866.9	865.9	0.12

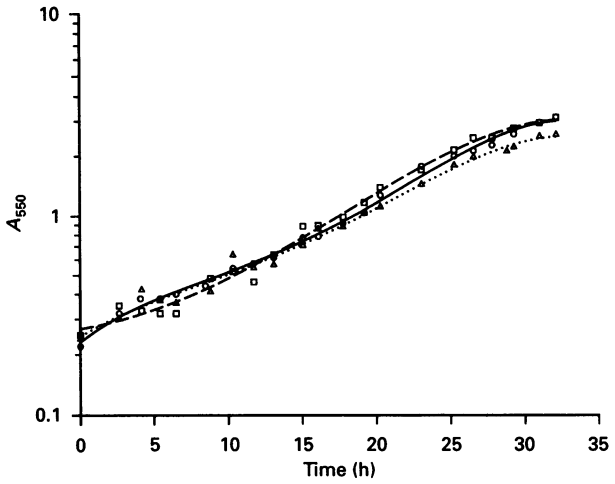

Figure 2 Growth curves for yeast with and without expression of recombinant bovine ACBP

○, pCGS109; □, pCGS109ACBP; △, pCGS109ACBP-T.

the *Bam*HI site and the *Sal*I site at position 1126. The vector sequence between *Sal*I (1126) and *Pst*I (2111) contains no consensus yeast terminator sequences (Zaret and Sherman, 1982) and transcripts from the *GAL1* promoter in pCGS109ACBP most probably terminate at the *URA3* terminator.

The stability of mRNA in yeast has been reported to decrease with increasing length of the mRNA (Brown et al., 1988). Thus, in order to possibly increase the stability of the ACBP mRNA, we constructed pCGS109ACBP-T where the *TRP1* terminator was inserted immediately 3′ of the synthetic ACBP gene.

Expression of bovine ACBP in yeast

The *GAL1* promoter is tightly regulated (Johnston and Davis, 1984; Yocum et al., 1984), and we were unable to detect any bovine ACBP in transformed yeast cells grown in the absence of galactose (results not shown). However, addition of galactose (20 g/l) to the growth medium resulted in a large induction of ACBP synthesis. In maximally induced exponentially growing cells containing the pCGS109ACBP or the pCGS109ACBP-T vector, bovine ACBP constitutes 1.8 % and 6.4 % of total soluble protein respectively. This indicates that insertion of the *TRP1* terminator, as expected, resulted in an increased stability of the ACBP transcript.

The high-level expression of bovine ACBP in yeast did not affect the growth rate when compared with control cells transformed with the vector without the ACBP gene inserted (Figure 2). This indicates that expression of bovine ACBP did not significantly affect the energy metabolism of the cells.

The expressed bovine ACBP was purified and the sequence was confirmed by peptide mapping with SAP followed by determination of the M_r of each individual peptide (Table 1). The M_r of the N-terminal SAP peptide could not be obtained. In order to confirm the primary structure of this part of the molecule, the protein was subjected to tryptic digestion and the mass of the N-terminal tryptic peptide (T1) was determined. The observed values of M_r deviated by no more than 0.14 % from the

Table 2 Content of the dominant acyl-CoA esters in yeast with and without expression of recombinant bovine ACBP

Values for acyl-CoA concentrations are means of double determinations ± half the difference. Values in parentheses show molar percentage distribution of each individual acyl-CoA. Note that 5×10^9 cells correspond to 500 A_{550}. For experimental details, see the Materials and methods section.

Plasmid	A_{550}	Acyl-CoA (nmol/5×10^9 cells) $C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	Total	ACBP (nmol/5×10^9 cells)
pCGS109	1.25	3.7±0.8 (50.0±6.0)	1.4±0.6 (17.0±2.0)	0.35±0.05 (5.0±1.0)	2.3±1.1 (28.0±5.0)	7.8±2.6	–
	1.5	2.1±0.2 (41.0±1.0)	0.55±0.05 (10.5±0.5)	0.45±0.05 (8.5±1.5)	2.05±0.15 (40.0±0)	5.2±0.4	–
pCGS109 ACBP	1.25	6.8±1.4 (57.0±4.0)	1.15±0.15 (10.0±0)	0.8±0.1 (7.0±0)	3.05±0.05 (26.5±3.5)	11.8±1.2	15.5±1.5
	1.5	7.05±1.15 (60.5±0.5)	1.3±0.3 (11.0±1.0)	0.5±0 (4.5±0.5)	2.8±0.4 (24.0±0)	11.7±1.9	14.0±0.8
pCGS109 ACBP-T	1.25	11.5±1.0 (63.5±5.5)	1.2±0 (7.0±0)	0.7±0 (4.0±0)	4.8±1 (26.5±5.5)	18.2±0.1	30.5±3.0
	1.5	13.0±0.3 (66.0±2.0)	0.7±0.1 (3.5±0.5)	1.6±0.2 (8.0±1.0)	4.45±0.65 (22.5±2.5)	19.8±1.1	30.3±2.3

expected values calculated from the sequence of bovine ACBP (Mikkelsen et al., 1987) (Table 1). The M_r obtained for the intact protein was 9955.9 which is in good agreement with the M_r calculated from the sequence (9955.3) (Mikkelsen et al., 1987). The M_r of the intact protein and the N-terminal tryptic peptide clearly showed that the processing of ACBP in yeast is identical with that of native bovine ACBP, i.e. the initiating methionine is removed and the following serine residue is *N*-acetylated. In contrast, when recombinant bovine ACBP is expressed in *E. coli*, the initiating methionine is removed but less than 5% of the following serine residue is *N*-acetylated (Mandrup et al., 1991; P. Højrup, unpublished work).

The acyl-CoA concentrations of cells expressing bovine ACBP (i.e. those transformed with pCGS109ACBP or pCGS109ACBP-T) were compared with the acyl-CoA concentrations of cells containing only the expression vector pCGS109. Expression of bovine ACBP in yeast resulted in a significant increase in the cellular acyl-CoA pool, and there was a positive correlation between the size of this increase and the level of ACBP expression (Table 2). The results shown in Table 2 are typical of results obtained in three individual experiments.

As the acyl-CoA synthetase (Suzue and Marcel, 1972; Oram et al., 1975) and the yeast fatty acid synthetase (Lust and Lynen, 1968) are product-inhibited, the only possible interpretation of these data is that ACBP, by binding acyl-CoA, relieves the product inhibition of these two enzymes and thereby induces increased synthesis of acyl-CoA. The binding stoichiometry has previously been found to be 1 mol of acyl-CoA/mol of ACBP (Knudsen et al., 1989). The molar increase in acyl-CoA concentration on expression of bovine ACBP in yeast amounts to 26–40% of the molar concentration of bovine ACBP. If all the acyl-CoA in the cells was bound to bovine ACBP under these conditions, ACBP would be between 61 and 84% saturated. Such a high level of saturation of ACBP with acyl-CoA is compatible with *in vitro* results showing that ACBP is able to bind long-chain acyl-CoA esters with high affinity and extract them from membranes (Rasmussen et al., 1990).

The main products of the yeast acyl-CoA synthetase are $C_{16:0}$ and $C_{18:0}$ acyl-CoA (Ratledge and Evans, 1989). Expression of bovine ACBP in yeast resulted in a significant increase in the relative contribution of $C_{16:0}$ to the total pool of acyl-CoA. On the contrary, the relative contributions of $C_{18:0}$ and $C_{18:1}$ were markedly decreased. This may indicate that ACBP, by removing acyl-CoA from the fatty acid synthetase, causes an earlier termination of the chain elongation.

The above data show that bovine ACBP can act as an intracellular acyl-CoA pool former in yeast cells. We therefore suggest that ACBP acts as an intracellular pool-forming and delivery system for long-chain acyl-CoA esters in eukaryotic cells.

We have recently determined the content of acyl-CoA in the liver of well-fed rats to be 25–30 nmol/g wet tissue (J. Rosendal, unpublished work). These values are similar to the ACBP concentration of rat liver (Knudsen et al., 1989), corroborating the notion that ACBP plays a major role in determining the size of the acyl-CoA pool

An additional function could be to protect membranes, enzymes and transport functions against damaging detergent effects of long-chain acyl-CoA esters. The mammalian mitochondrial ATP/ADP translocase and acetyl-CoA carboxylase, both of which are inhibited at nanomolar concentrations of acyl-CoA (Morel et al., 1974; Nikawa et al., 1979), would be completely inhibited if acyl-CoA was not effectively sequestered. We have previously shown that ACBP binds acyl-CoA esters with high affinity and prevents binding to microsomal membranes (Rasmussen et al., 1990). These data, together with the results obtained in the present work, strongly indicate that ACBP *in vivo* protects the above-mentioned functions from being inhibited by acyl-CoA esters.

ACBP could therefore play a triple role in the cell in (1) creating an acyl-CoA pool, (2) protecting membranes, transport functions and enzymes and (3) protecting acyl-CoA esters from being hydrolysed by long-chain acyl-CoA hydrolases which are found in high concentration in the liver cytosol and microsomes (Berge, 1979; Berge and Farstad, 1979; Berge et al., 1984).

Furthermore, ACBP may be involved in delivering acyl-CoA esters for protein acylation. Immunohistochemical studies have shown that cells involved in secretion and other transport processes are particularly rich in ACBP (Bovolin et al., 1990), indicating that ACBP may be associated with such processes. Acyl-CoA esters (probably used for protein acylation) have recently been shown to be obligatory for the budding off of transport vesicles in Golgi membranes as well as for the fusion of these vesicles with their acceptor membranes in the Golgi apparatus (Pfanner et al., 1989, 1990). ACBP may be involved in delivering acyl-CoA esters for this purpose.

In summary, the present work gives conclusive evidence that the physiological function of ACBP is related to acyl-CoA metabolism. The ability of ACBP to function as an acyl-CoA pool former *in vivo* is in accordance with the detection of ACBP transcripts (Mochetti et al., 1986; Gray et al., 1986) and protein (Mikkelsen et al., 1987; Shoyab et al., 1986; Knudsen et al., 1989; Bovolin et al., 1990; Alho et al., 1991) in most tissues and many different cell types and with the recent observation that the rat ACBP gene is a typical housekeeping gene (Mandrup et al., 1992). The claimed functions of ACBP in the regulation of steroidogenesis (Bessman et al., 1989; Papadopoulos et al., 1991), in the modulation of the $GABA_A$ receptors (Costa and Guidotti, 1991) and in the inhibition of glucose-induced insulin secretion (Östenson et al., 1990) do not require that ACBP should be widely expressed. Furthermore, these claimed functions do not explain why the expression of ACBP should be significantly increased on induction of preadipocyte-to-adipocyte differentiation of 3T3-L1 cells, as we have shown previously (Hansen et al., 1991). Thus, although we cannot exclude the possibility that ACBP executes specific tasks in specialized cells, there seems to be no doubt that it is primarily a housekeeping protein, functioning as an acyl-CoA pool former and transporter.

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