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REGULATION OF ATP-CITRATE LYASE AT TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL LEVELS IN RAT LIVER

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The amounts of ATP-citrate lyase in liver cytosol began to increase at 12 hours after refeeding a high-carbohydrate diet and further increased until 48 hours. The amounts of the ATP-citrate lyase mRNA began to increase at 6 hours and reached to a maximum level at 12 hours, followed by decrease to a very low level until 48 hours. The elevated amount of the ATP-citrate lyase mRNA reflected on the increase of ATP-citrate lyase content in the first 24 hours, but these two parameters were not paralleled thereafter. The transcriptional activity of ATP-citrate lyase gene in nuclei of rat liver began to increase at 4 hours and further increased to reach a maximum level of 24 fold at 12 hours, maintaining a high level of 17 fold until 48 hours. The elevation of transcriptional activity of ATP-citrate lyase gene preceded the increase of ATP-citrate lyase mRNA content in the liver cytosol by 2 hours, and its increasing pattern was similar to changes of mRNA content until 12 hours. However, while the transcriptional activity remained at a high level until 48 hours, the ATP-citrate lyase mRNA concentration in the cytosol decreased after 12 hours.

Animal ATP-citrate lyase (EC 4.1.3.8) is a cytosolic enzyme that plays a role of transfer of acetyl CoA from mitochondria to cytosol for the synthesis of fatty acid and cholesterol, using citrate as a carrier molecule. The reaction catalyzed by this enzyme is:

$$\text{Citrate} + \text{ATP} + \text{CoA} \xrightarrow{Mg^{2+}} \text{Acetyl CoA} + \text{ADP} + \text{Pi} + \text{Oxaloacetate}$$

ATP-citrate lyase in the rat liver is known to consist of four identical subunits of which molecular weight is about 110,000 (1,2). The content of ATP-citrate lyase is known to decline sharply by starvation and to increase by refeeding a high-carbohydrate diet after starvation(3). It has been reported that the induction of ATP-citrate lyase by refeeding corresponds to the increase of its mRNA content (4). Although a number of studies on nutritional and hormonal regulation of ATP-citrate lyase have been reported, the enzyme induction and its mRNA contents are independently measured in each study, and the changes in transcriptional activity of ATP-citrate lyase gene remain unknown. In the present study, the changes of the transcriptional rate on ATP-citrate lyase gene, the ATP-citrate lyase mRNA concentration, and the enzyme concentration were measured in rat

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livers at various times after refeeding a high-carbohydrate diet *ad libitum* to fasted rats, in order to determine the relationships between these parameters.

MATERIALS AND METHODS

Male Sprague-Dawley rats (150 to 200 g) were fasted for 72 hours and then refed a high-carbohydrate diet at 8 a.m.. At each indicated time, the rats were decapitated and the livers were isolated after the perfusion with normal saline into portal vein in order to remove the blood. The cytosolic protein, RNA and nuclei were isolated from five pooled liver tissues.

ATP-citrate lyase cDNA's used in this study were pGACL5 and pGACL9C, which were previously cloned (5), and pGACL1 prepared by polymerase chain reactions (PCR). PCR was performed as described by Kawasaki (6), using poly(A)-rich RNA isolated from rat liver. The upstream primer at position 550 to 570 (TCATCTCCGGCCTATTCAAT) and downstream primer at position 2100 to 2080 (CAGCACGTGATCCATGAATGT) were synthesized according to the sequence of ATP-citrate lyase cDNA (7), using Applied Biosystems 381A DNA synthesizer. The thermal cycle was denaturation for 30 seconds at 95°C, annealing for 30 seconds at 55°C and extension by Taq polymerase for 30 seconds at 72°C. After 30 thermal cycles, the reaction mixture was finally incubated for 10 minutes at 72°C for the extension. The pGACL1 was constructed by the ligation of amplified cDNA to pGACL5 digested with KpnI and BglII (Fig.1.). The pGACL2 was constructed by insertion of 0.73 kb cDNA of pGACL9 into EcoRI site of pGACL1 (Fig.1.).

The ATP-citrate lyase content in rat liver was determined by Western blot. The rat liver soluble protein fraction was prepared from 10 ml of 10% homogenate by centrifugation at 15,000 xg for 10 min. The concentration of protein was determined by the methods of Bradford (8). The protein (100 μg) was fractionated on 5% SDS polyacrylamide gel (9) and transferred to nitrocellulose membrane (10). The ATP-citrate lyase band on nitrocellulose membrane was immunostained with a combination of polyclonal anti-ATP-citrate lyase antiserum and anti-rabbit IgG-alkaline phosphatase conjugate (11).

The ATP-citrate lyase mRNA content was measured by Northern analysis. Total RNA was isolated from the rat livers, according to MacDonald *et al.* (12). The ratio of A_{260} to A_{280} of isolated RNA was 1.8-2.1 and the concentration of RNA was determined by the measurement of the absorbance at 260 nm. Northern analysis was performed as described by Sambrook *et al.* (13). Total RNA (20 μg) was electrophoresed on 0.8% agarose gel containing 2.2M formaldehyde and transferred to the nitrocellulose membrane (2μm). The membrane was probed with [^{32}P]-labeled ATP-citrate lyase cDNA of pGACL9 and then exposed to Fuji RX film with Du Pont Lighting-Plus intensifying screens at -70°C for 16 hours.

The run-on transcription in rat liver nuclei was performed as described by Tilghman and Belayew (14). The run-on transcripts were hybridized to pGACL2 and β-actin, blotted to nitrocellulose membrane. After hybridization, filters were washed for 1 hour in 2x SSC at 65°C. The filters were then incubated at 37°C in 2x SSC with RNase A (10 μg/ml) for 30 minutes and were subsequently washed for 1 hours. Filters were then exposed to Fuji RX film in cassettes containing intensifying screen at -70°C for 72 hours. To quantify the amount of RNA bound to each slot of cDNA, the autoradiographic image of the slot was scanned using a densitometer. The ratio of the optical density of ACL cDNA to that of β-actin was calculated and normalized to the value of fasted group (at zero time).

RESULTS AND DISCUSSION

The partial cDNA's for ATP-citrate lyase, pGACL5 and pGACL9 were previously obtained by screening a rat liver λgt11 cDNA library using anti-ATP-citrate lyase antibody

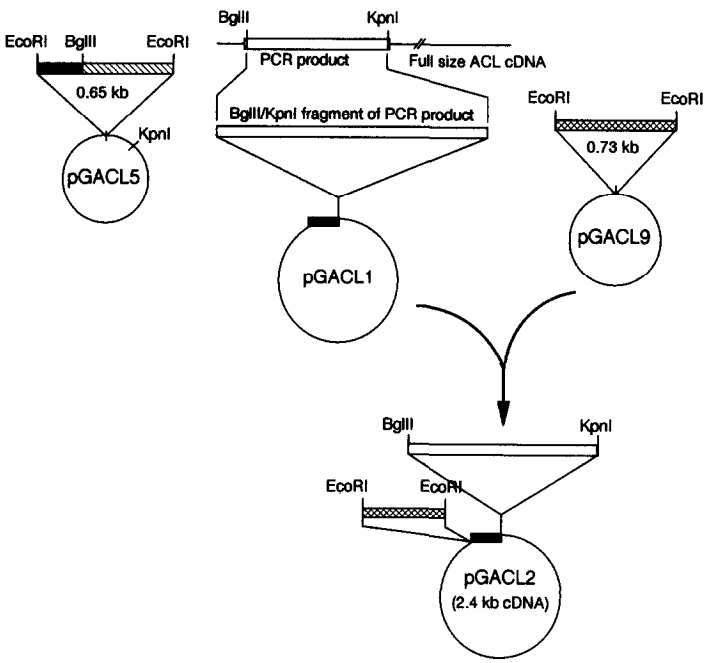

Fig. 1. Schematic diagram of construction of pGACL1 and pGACL2.
The cDNA amplified by PCR was digested with BglII and KpnI and joined with pGACL5 at BglII and KpnI restriction sites. The resulting plasmid was named as pGACL1. The 0.73 kb EcoRI fragment from pGACL9 was joined with pGACL1 at EcoRI restriction site and named as pGACL2.

(5). The size of the cDNA of pGACL5 and pGACL9 was 0.65 and 0.73 kb, respectively. However, it was necessary to obtain a longer cDNA in order to increase the hybridization signal in run-on transcription assay. To this end, PCR was performed with upstream (550 to 570) and downstream (2083 to 2103) primers (7). The amplified cDNA showed 1.55kb in length on agarose gel electrophoresis, and its size was slightly reduced when digested with KpnI and BglII (Fig.2.A.). The plasmid pGACL1 was prepared by insertion of amplified cDNA into pGACL5 digested with KpnI and BglII. The restriction site deduced by the sequence in pGACL1 were confirmed (Fig.2.B.) and its entire nucleotide sequence was revealed (data not shown). By this procedure, we could obtain a large ATP-citrate lyase cDNA.

The uptake of a low-fat/high-carbohydrate diet after starvation has been known to increase the biosynthesis of the lipogenic enzymes (15). ATP-citrate lyase providing the cytosolic acetyl CoA for fatty acid synthesis has been also reported to be induced by a high-carbohydrate diet (3). However, measurements of the induction of ATP-citrate lyase protein and its mRNA were independently performed in separate studies and its transcriptional activity in nucleus has not been assayed, yet. Consequently the sequential events of this enzyme induction at transcriptional and post-transcriptional level remain

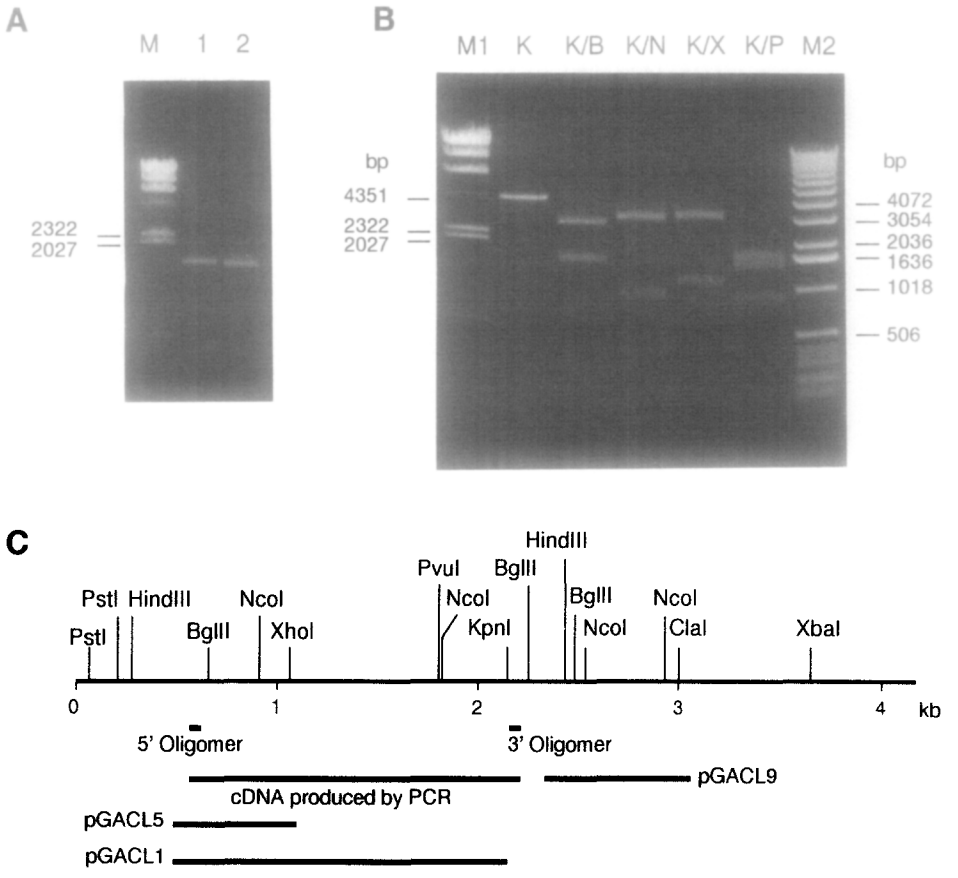

Fig. 2. Agarose gel electrophoresis and the restriction map of ATP-citrate lyase cDNA. Panel A shows the agarose gel electrophoretic pattern of PCR product. Lane M, 1, and 2 show the molecular weight size marker, 0.1 μg of PCR product, and PCR product digested with KpnI and BglII, respectively. Panel B shows the agarose gel electrophoretic pattern of pGACL1 after digestion with restriction enzymes. Lane M1 and M2 show the molecular weight size marker of Lambda-HindIII digest and 1 kb ladder, respectively. Lane K, K/B, K/N, K/X, and K/P show the restriction fragments of pGACL1 after digestion with following enzymes: KpnI, KpnI and BglII, KpnI and NcoI, KpnI and XhoI, and KpnI and PvuI, respectively. Panel C shows the scheme of the full cDNA clone of ATP-citrate lyase(7). The locations of the two PCR primers used for amplification are indicated by short lines. pGACL1 cloned by PCR and pGACL5 and pGACL9 cloned by screening the cDNA library (5) are indicated.

unknown. We have therefore compared the time courses of ATP-citrate lyase protein induction with its mRNA contents and transcriptional rates in nuclei.

The level of ATP-citrate lyase protein was rapidly increased at 12 hours after refeeding, and it was further continuously increased until 48 hours (Fig.3.). The time course of ATP-citrate lyase enzyme induction resembled that of fatty acid synthase (16), acetyl CoA carboxylase (17), malic enzyme (18) and glucose-6-phosphate dehydrogenase (18,19).

The concentration of ATP-citrate lyase mRNA began to increase after 6 hours and reached the plateau at 12 hours (Fig.4.). But thereafter, ATP-citrate lyase mRNA was markedly decreased to a very low level until 48 hours (Fig.4.). Although the initial

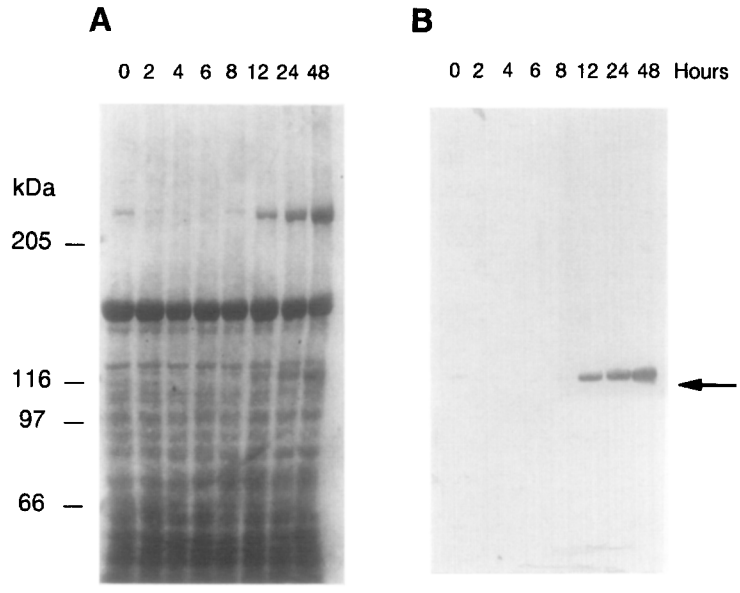

Fig. 3. Western blot analysis of ATP-citrate lyase after refeeding a high-carbohydrate diet.
After refeeding a low-fat/high-carbohydrate diet to fasted rats, time courses for the amount of hepatic ATP-citrate lyase were followed. The rats were killed at the times indicated on the top of the figure, after refeeding. The protein (100 μg) from liver homogenate obtained at each time was fractionated on 5% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane, followed by immunostaining with affinity-purified anti-ATP-citrate lyase IgG and anti-rabbit IgG-alkaline phosphatase conjugate. The standard molecular weight is shown at the left in kDa. Panel A shows the electrophoretic pattern after protein staining with Coomassie, and panel B shows the nitrocellulose membrane after immunostaining. The arrow indicates the position of immunoreactive ATP-citrate lyase.

induction of ATP-citrate lyase protein until 24 hours was corresponded to the increase of its mRNA, there was a difference in the changes of these two parameters, between 24 and 48 hours. Between 12 and 24 hours, the enzyme induction could be explained by accumulation of protein due to its long half-life in spite of the decrease of its mRNA content. However, the increase of enzyme content after 24 hours was thought to be resulted from the increase of translational efficiency rather than from the increase of mRNA, because the ATP-citrate lyase mRNA content decreased to a markedly low level during this period. It has been reported that the concentration of fatty acid synthase mRNA reached the maximum level 16 hours after refeeding, and this increased level gradually decreased until 72 hours (16). Iritani *et al.* (20) reported that in the cases of malic enzymes and glucose-6-phosphate dehydrogenase, their mRNA concentrations reached a maximum at 16 hours after refeeding and the elevated level maintained until 72 hours. The time courses of the concentration of ATP-citrate lyase mRNA by refeeding showed a similar pattern to those of

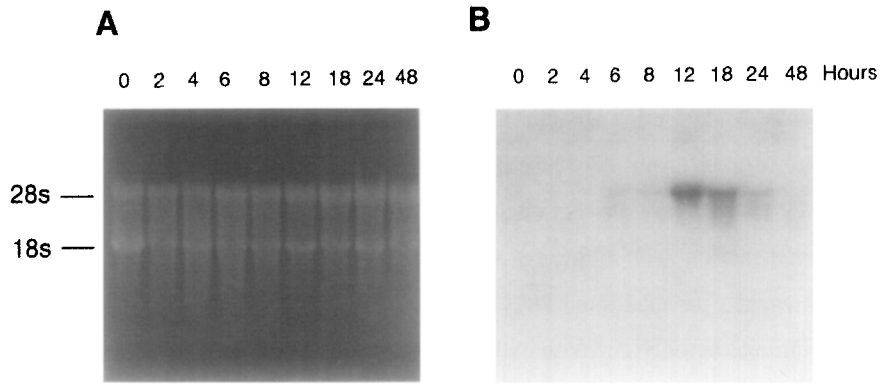

Fig.4. Northern analysis of RNA in rat liver after refeeding a high-carbohydrate diet. After refeeding a low-fat/high-carbohydrate diet to fasted rats, total cytoplasmic RNA were isolated using guanidine-HCl and separated on a 1% agarose gel containing 2.2 M formaldehyde. The RNA was then transferred to a nitrocellulose membrane and hybridized with the [^{32}P]-labeled pGACL9 cDNA. Each lane contains 20 μg of total RNA from livers at each time course of refeeding. The times after refeeding were shown on the top of the lanes. Panel A shows the agarose gel electrophoretic pattern of total RNA and panel B shows the autoradiogram after hybridization with ATP-citrate lyase cDNA probe.

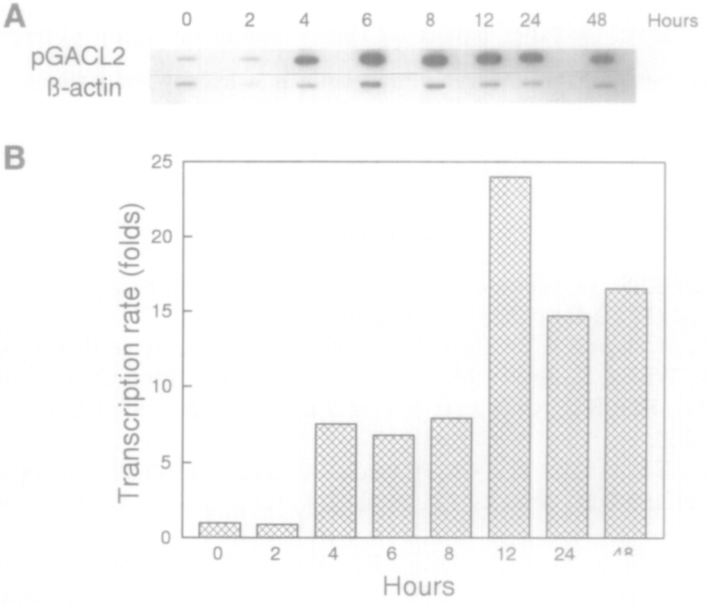

Fig. 5. The effects of refeeding on the transcriptional activities of ATP-citrate lyase gene in rat liver nuclei.
The run-on transcripts in nuclei isolated from livers at each time after refeeding were hybridized for 36 hours to β-actin and pGACL2 and blotted onto the nitrocellulose membrane. After hybridization, the nitrocellulose membrane was washed and autoradiographed at -70°C for 72 hours (panel A). Panel B shows the relative transcriptional activities of the ATP-citrate lyase gene.

fatty acid synthase of other lipogenic enzymes, but the increased level was more rapidly decreased to a basal level.

In order to determine whether the dramatic changes in ATP-citrate lyase mRNA content were resulted from the transcription on its gene, we measured the transcriptional rates on ATP-citrate lyase gene in nuclei. By refeeding a high-carbohydrate diet, the transcriptional rate on ATP-citrate lyase gene began to increase about 7.5 folds after 4 hours, and reached the maximum level of 24 folds after 12 hours (Fig.5.). After 12 hours, the transcriptional activity decreased slightly but maintained a very high level of 17 folds until 48 hours (Fig.5.). The patterns of the changes in transcriptional rate on ATP-citrate lyase gene by refeeding were similar to other lipogenic enzymes such as fatty acid synthase, acetyl Co carboxylase , malic enzyme and glucose-6-phosphate dehydrogenase (16,17,20). The initial increase of ATP-citrate lyase mRNA until 12 hours was reflected by the increased level of transcriptional rate on ATP-citrate lyase gene. However the decline of concentration of ATP-citrate lyase mRNA after 12 hours seems to be due to the changes of stabilization and/or processing of its transcripts, rather than due to the decrease of transcriptional activity.

The present observations suggests that initial phase of ATP-citrate lyase induction by refeeding a high-carbohydrate diet correlates to the increase of mRNA concentration and transcription rate, but thereafter another regulatory machineries seems to be present at post-transcriptional level, such as changes of translational efficiency and stability and/or processing of ATP-citrate lyase transcripts.

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