Differential Expression Pattern of S-Adenosylmethionine Synthetase Isoenzymes During Rat Liver Development

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The pattern of expression of liver-specific and extrahepatic S-adenosylmethionine (SAM) synthetase in developing rat liver was established by determining steady-state levels of the respective messenger RNAs (mRNAs) and protein content. Levels of liver-specific SAM synthetase mRNA increased progressively from day 20 of gestation, increased 10-fold immediately after birth, and reached a peak at 10 days of age, decreasing slightly by adulthood. Conversely, mRNA levels of extrahepatic isozyme decreased toward birth, increased threefold in the newborn, and decreased further in the postnatal life, reaching a minimum in the adult. Similar expression profiles were observed in isolated hepatocytes, indicating that both mRNAs are differentially regulated in the same cell type. Western blot analysis showed that levels of immunoreactive liver-specific isoform followed a trend similar to the mRNA, indicating that developmental regulation of this enzyme is mediated at the mRNA level. Developmental patterns of expression of albumin and α-fetoprotein (AFP) mRNAs were closely related to those for liver-specific and extrahepatic isoenzymes, respectively. Therefore, it is suggested that liver-specific SAM synthetase may be a marker for hepatocyte differentiation. Incubation of primary cultures of hepatocytes from 21-day-old fetuses with permeant cyclic adenosine monophosphate (cAMP) analogues elicited an up-regulation of the mRNA for the liver-specific isoform with a concomitant down-regulation of the extrahepatic message, suggesting a physiological role for the increased postnatal glucagonemia in the control of this isoenzyme switching. In contrast with the isoenzyme expression profiles, the levels of SAM, the product of SAM synthetase reaction, were determined to be greater during gestation than in immediate postnatal periods. These results indicate that synthesis and utilization of SAM may be regulated differentially in fetal and adult hepatocytes. (HEPATOLOGY 1996;24:876-881.)

Growth and development of mammalian liver proceed with characteristic alterations of the enzyme pool through which it acquires the capability of coping with the demands of altered environmental conditions imposed by birth and postnatal life. This process, which involves the expression of new enzymes and changes in the concentration of those already present, is known as enzymic differentiation and concerns a number of enzymes accounting for main liver functions, among them, S-adenosylmethionine (SAM) synthetase.

SAM synthetase (EC 2.5.1.6) catalyzes the only known biosynthetic route to SAM, the major donor of methyl groups, from adenosine triphosphate and methionine. This reaction is especially important in the liver, where as much as 48% of the methionine taken by the diet is metabolized and up to 85% of all transmethylation reactions occur. According to its crucial role in this organ, mammals express a liver-specific SAM synthetase isoenzyme, whereas in other tissues, the so-called extrahepatic or kidney-type SAM synthetase is present (reviewed by Kotb and Geller and Mato et al.). The functional relevance of this pattern of expression of SAM synthetase remains unclear, although some suggestions have been addressed. These isoenzymes seem to be encoded by different genes, as judged by sequence comparison of the corresponding complementary DNAs (cDNAs).

Earlier studies showed that marked changes in SAM synthetase activity occur during liver development. Thus, in mouse liver, Hancock reported a rapid increase in SAM synthetase activity after birth from trace activities in the near-term fetus to a peak value at the age of 21 days. Finkelstein also reported a significantly greater SAM synthetase activity in the suckling and weaning rat than in the adult. In a comprehensive study, Chase et al. found that specific activity began to increase in late fetal life, reaching a maximum 2 days after birth and decreasing slightly by adulthood. Changes in SAM synthetase isoenzymes during development have also been reported. Based on their respective kinetic properties and on immunohistochemical analysis, it has been shown that rat liver-specific SAM synthetase is expressed only in adult liver, whereas the extrahepatic isoform is predominantly expressed in fetal liver and faintly detected in the adult organ. In humans, it has also been reported that the extrahepatic form is weakly expressed in adult liver, and in contrast to the rat, the liver-specific form is present at very low levels in fetal liver.

Altogether, the data available suggest that a SAM synthetase isoenzyme switching occurs during liver development. To date, however, a comprehensive survey of developmental profile of these isoenzymes has not been performed. In the present study, we have delineated the developmental pattern of messenger RNA (mRNA) expression of both SAM synthetases in rat liver. Special attention has been paid to the mechanism that controls this switch of isoenzymes and to the levels of SAM at different stages of liver development.

MATERIALS AND METHODS

Animals. Pregnant, 3-month-old, albino Wistar rats (weight range, 300-350 g) were fed a standard laboratory diet and were killed for the experiment between 9 and 10 AM. Gestation age was assessed following standard criteria. Fetuses of the indicated age were delivered by cesarean section after the mother was anesthetized with...
Nembrital (Abbott Labs, North Chicago, IL). Animals were treated following the institution’s criteria for the care and use of laboratory animals.

**Isolation of Fetal, Neonatal, and Adult Hepatocytes.** Fetal and neonatal liver hepatocytes were prepared by a nonperfusion collagenase dispersion method. Briefly, the tissue was chopped with scissors and incubated for 30 minutes at 37°C with Ca²⁺-free Krebs-bicarbonate buffer containing 0.5 mM/L ethylene glycol-bis (β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA) and 0.1 mg/mL streptomycin. Fetal hepatocytes (3-4 x 10⁶) were plated in 6-cm tissue-culture dishes in 2.5 mL of Dulbecco’s modified Eagle medium supplemented with 10% fetal calf serum. After 4 hours of incubation to facilitate cell attachment to the plate, the medium was aspirated, the plates were washed twice with phosphate-buffered saline to remove the nonadherent cells and filled with Dulbecco’s modified Eagle medium, containing 10 mg/mL of fatty acid–free bovine serum albumin. Hormones additions were made so that the changes in the total incubation volume were <2%. Dexamethasone and insulin were added to a final concentration of 1 μM/L; prolactin was added at 100 ng/mL, and ph–cyclic adenosine monophosphate (cAMP) plus theophylline were added at 100 μM/L. Hormones and other agents were obtained from Novo Industri A/S (Copenhagen, Denmark), Sigma Chemical Co. (St. Louis, MO), and Merk AG (Darmstadt, Germany).

**cDNA Probes.** A cDNA comprising 1.147 base pairs of the rat extrahepatic SAM synthetase was obtained by reverse-transcription coupled to polymerase chain reaction. Primers were designed according to the published cDNA sequence. The first cDNA strand was synthesized from 3 μg of rat kidney Poly (A) (Clontech, Palo Alto, CA) RNA using 200 U of Moloney murine leukemia virus reverse-transcriptase (Superscript II; GIBCO BRL, Paisley, Scotland) and the downstream primer (5′-AGCTGTCCCTACCAAAGTGGC-3′), complementary to nucleotide residues 1133-1153 of the extrahepatic SAM synthetase sequence. The cDNA was then subjected to polymerase chain reaction amplification with the above primer and the upstream primer (5′-AAAGCTTTCTCAGGAAGTGC-3′), corresponding to nucleotides 6-26. Amplification was performed as described previously but using the thermostable DNA polymerase Dynazyme (Finnzymes, Oy, Finland). The resulting fragment was purified, inserted into the Smal site of pUC18 plasmid, and sequenced by the dideoxy chain termination method to confirm its authenticity. Other cDNA fragments used as probes were as follows: a 2.2-kilobase EcoRI fragment of the rat liver-specific SAM synthetase cDNA SSRL²; a fragment comprising 1.1 kilobases of rat albumin cDNA clone pRSA13; a 0.6-kilobase fragment of the cDNA for the rat α-feto-protein (AFP)²; and a cDNA for the 18S ribosomal RNA inserted into plasmid pBR322 was used for normalization.

**Northern Blot Analysis.** Total RNA was isolated by the guanidine isothiocyanate method. Total RNA (~30 μg) was fractionated on a 0.9% agarose denaturing gel and transferred to Nytran membranes (Schleicher and Schuell, Keene, NH). Prehybridization and hybridization were performed as described previously. The probes were random primed labeled with [α-³²P]deoxycytidine triphosphate using the Readyprime labeling kit (Amersham Ltd, Little Chalfont, England). The filters were scanned on a Molecular Imager GS-250 (Bio-Rad Laboratories, Richmond, CA). Protein quantitation was performed by a densitometric analysis using the Phosphor analyzer software (Bio-Rad Laboratories).

**Western Blot Analysis.** Samples of rat liver or hepatocytes were homogenized in 10 mM/L Tris-HCl, pH 7.5, and 0.3 mM/L sucrose buffer. Twenty micrograms of proteins from the cytosolic fractions were run on sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels and electrophoresed as described previously. Proteins were electrotransferred to nitrocellulose membranes using 20 mM/L of Tris-HCl, pH 7.5, containing 20% methanol. SAM synthetase was detected by using a rabbit antiserum raised against purified liver-specific SAM synthetase and goat anti-rabbit IgG horseradish peroxidase conjugate (Bio Rad, Richmond, CA) antibody.

**RESULTS**

The pattern of expression of both liver-specific and extrahepatic SAM synthetase in developing rat liver was determined by Northern blot, using specific cDNA probes. As previously reported, the liver-specific cDNA probe failed to cross-hybridize with the mRNA for the extrahepatic isoenzyme and vice versa. As shown in Fig. 1, the content of liver-specific SAM synthetase mRNA detected in pooled livers derived from animals of 20-22 days of gestation is rather low, although it increases progressively until birth. Immediately after birth, the rate of increase is quite sharp, resulting in an approximately 10-fold change in the mRNA levels. These levels then remain constant at least until 10 days of age but decrease slightly by adulthood. The expression profile shown by the extrahepatic SAM synthetase mRNA falls into a markedly different pattern. The highest levels are detected at day 20 of gestation and decrease toward birth, increasing threefold in the newborn. Subsequently, a rapid reduction in the mRNA content is observed 3 hours after birth, returning to the value measured the last day of gestation. Then, it remains essentially constant throughout the first 20 days of postnatal life, reaching the lowest concentration in the adult rat. These results were reproduced without significant variations in four independent experiments. It is worth noting that blot hybridized with the extrahepatic SAM synthetase probe were subjected to an exposure 10 times longer than when hybridized with the liver-specific SAM synthetase probe, although the specificity of both probes was similar. It is therefore concluded that, in general, outstanding differences exist in net expression of both mRNAs, and these differences enlarge throughout development, as judged by the intensity ratio of the corresponding signals (Fig. 1B).

Albumin and AFP levels were also determined as a control for the assessment of the developmental status of the tissue. The expression of both mRNAs followed the expected pattern. Thus, albumin mRNA content increased in late fetal life and persisted to adulthood at comparable levels. The amount of AFP also increased throughout the late fetal period but decreased after birth and became undetectable at day 20 of postnatal life. It is interesting to note that liver-specific SAM synthetase could be a reliable indicator of the differenti-
rats closely resembles that found in the total liver, except for AFP, whose levels appear to be relatively greater 3 days before birth. The intensity ratio of the signals yielded by liver-specific and extrahepatic SAM synthetase mRNA bands in hepatocytes (Fig. 2B) is also similar to that observed in the total liver. Therefore, it is concluded that changes in the developmental program of both mRNAs occur in the same cell type.

To further define the molecular basis for the regulation of liver-specific SAM synthetase in different developmental stages, we also determined the content of the specific protein. Extracts of cytosolic proteins derived from aliquots of the same samples used for Northern analysis were subjected to immunoblotting. As shown in Fig. 3, changes in the specific protein during development show a trend similar to the corre-

![Fig. 1](image1.png)

**Fig. 1.** Developmental regulation of liver-specific and extrahepatic SAM synthetase mRNAs in rat liver. (A) Northern blot of total RNA (30 μg) derived from rat livers at the indicated stages of development. For fetal and newborn stages, livers were pooled from several individuals. (A) Signal obtained after hybridization of the membrane with [32P]cDNA probe for liver-specific SAM synthetase. A longer exposure of the lanes corresponding to fetal stages is shown below. The same blot was hybridized subsequently to extrahepatic SAM synthetase, albumin, and AFP probes. The exposure period of the blot after hybridization with the extrahepatic SAM synthetase probe was 10 times longer than for the corresponding liver-specific SAM synthetase probe. (B) The intensity ratio of liver-specific SAM synthetase/albumin (□) and liver-specific SAM synthetase/albumin (□) mRNA bands obtained by densitometric scanning of the blots and normalized against 18S ribosomal RNA. Values are expressed as the percentage relative to the point that yielded the highest ratio. Statistical significance is as follows: *P* < .001, prenatal days vs. postnatal period. The figure reflects a typical result representative of four independent experiments.

![Fig. 2](image2.png)

**Fig. 2.** (A) Northern blot prepared from equal amounts (30 μg) of total RNA from isolated rat hepatocytes at different developmental stages was hybridized sequentially with the indicated probes. The time of exposure of the blots was as detailed in Fig. 1. (B) Intensity ratio of liver-specific SAM synthetase/extrahepatic SAM synthetase mRNA bands. Results shown are representative of four experiments.
liver or (B) rat hepatocytes at the indicated developmental stages were frac-

ted in the content of extrahepatic mRNA in total liver.

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cific SAM synthetase at different developmental stages in total liver (Fig.

to Western analysis. (D) Densitometric analysis of immunoreactive liver-spe-

theticase mRNA in fetal rat liver differ from previous studies and newborns is significantly greater than that measured in

the immediate postnatal periods (P < .05) and similar to the

one detected in adult rat.

DISCUSSION

In the present study, we have delineated the development-

mental patterns of expression of liver-specific and extrahe-

patic SAM synthetases by determining the respective mRNA levels. This approach was followed as an accurate way to
discriminate both isoenzymes, bearing also in mind that changes in the production of proteins during normal develop-

ment are controlled primarily at the level of mRNA synthesis.

Our results show that a switch in the predominant expres-
sion from the extrahepatic to the liver-specific isoenzyme oc-
curs in rat liver in late fetal life. The concentration of the

mRNA for the liver-specific SAM synthetase increases from

the earliest fetal age studied, increases strikingly after birth,

and peaks at age 10 days. Conversely, mRNA levels of the

extrahepatic isoenzyme decrease gradually until birth and

reach a minimum in the adult life. Interestingly, isolated

hepatocytes mimic isoform switching, indicating that both

mRNAs are differentially regulated in the same cell type.

This finding also excludes the possibility that changes ob-
served in the content of extrahepatic mRNA in total liver
could be caused by the contribution of hematopoietic cells

present in different proportions at each fetal stage.

Results concerning the presence of liver-specific SAM syn-

thetase mRNA in fetal rat liver differ from previous studies

that suggested a lack of expression of this isoenzyme at this
developmental stage.18,19 This discrepancy may be caused by

the different methods used to detect the respective isoen-

zymes. Another possibility would be that formation of the

different isoenzymes observed after birth. As shown in Fig.

4, when fetal hepatocytes were incubated with a permeant

analogue of cAMP, an important up-regulation of the liver-
specific SAM synthetase concomitant with a down-regulation

of the extrahepatic isoenzyme was observed. When hepato-
cyes were incubated with insulin, even at concentrations

that involve the occupation of the insulin or insulin-like

growth factor receptor dexamethasone or prolactin, the be-

havior of the two messages was the same as in untreated

cells. It is of interest to mention that on culture, fetal hepato-
cyes showed a progressive loss of the mRNA levels for the

liver-specific enzyme, which again argues in the sense of an

increased expression of this specific isoenzyme as hepatocyte

maturation and differentiation develops.

Altogether, the results described above are consistent with

the developmental patterns of SAM synthetase activity de-

scribed previously.15,17 To determine whether these changes

also correlate with SAM content, the levels of this metabolite

during different developmental stages were measured (Fig. 5).

Unexpectedly, the concentration of hepatic SAM in fetuses

and newborns is significantly greater than that measured in

the immediate postnatal periods (P < .05) and similar to the

one detected in adult rat.

responding mRNA expression either in total liver or in hepato-
cyes. The antibody raised against the liver-specific SAM syn-

thetase did not cross-react with the extrahepatic form

because no signal was detected either in kidney or spleen

(Fig. 3C), two tissues where only this isoenzyme is expressed.

The marked accumulation of liver-specific SAM synthetase

observed immediately after birth suggests that the hormonal

changes characteristic of neonatal animals could be the fac-

tors responsible for this cut-off switch. An important gluca-
gonemia associated with a decrease in the insulin levels has

been considered one of the factors that trigger the regulation

of gluconeogenic enzymes.35 In addition to these hormones,

prolactin reaches a peak in the newborn.36 Therefore, we
decided to investigate whether by using an ex vivo system of

cultured fetal hepatocytes (21 days of gestation) these hor-

mones could reproduce the expression patterns of SAM syn-
thetase isoenzymes observed after birth. As shown in Fig.
FIG. 4. Effect of hormones on liver-specific and extrahepatic SAM synthetase mRNAs in primary cultures of fetal hepatocytes. Hepatocytes from 21-day-old fetuses were cultured as detailed in Materials and Methods and were incubated for 3, 6, and 18 hours in the absence or presence of the indicated effectors. (A) Northern blot analysis performed with 30 μg of total RNA. Filters were hybridized sequentially with liver-specific and extrahepatic SAM synthetase probes. The time of exposure was as indicated in Fig. 1. (B) Intensity ratio of liver-specific SAM synthetase/extrahepatic SAM synthetase mRNA bands after normalization against 18S ribosomal RNA. Results are expressed relative to the value at time zero, which was taken as 100%. The figure reflects a representative experiment of three experiments. (○), Control; (○), dexamethasone; (×), prolactin; (●), insulin; (□), ph-cAMP.

An interesting finding is that liver-specific SAM synthetase expression parallels the switch on of the albumin gene, showing a notorious increase around delivery that remains stable up until the adult life. Also, changes in development in AFP and liver-specific SAM synthetase mRNAs either in total liver or in hepatocytes occur in opposite directions. These results suggest that liver-specific SAM synthetase could be a trait of the differentiated state of the hepatocyte. Related to this, it is worth noting that hepatocyte culturing is accompanied by a loss of the liver-specific isoenzyme mRNA, an effect readily attributed to the classic phenomenon of dedifferentiation of primary cultures. It also should be mentioned that, in a dedifferentiated hepatic cell line such as rat hepatoma H35, the levels of the liver-specific SAM synthetase are comparatively much lower than in hepatocytes (Gil B, et al., Unpublished observation, October, 1995).

The necessity of modulating SAM synthetase expression as a function of development must be oriented to cope with the changing demands of SAM. In this context, intriguing questions derive from the finding that, in contrast to the overall SAM synthetase expression pattern, levels of SAM are significantly greater in late gestation and after birth than in immediate postnatal periods. This may be interpreted in the light of previous studies performed in humans and...
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