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DEVELOPMENTAL AND GROWTH-RELATED REGULATION OF EXPRESSION OF SERINE DEHYDRATASE mRNA IN RAT LIVER[1]

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SUMMARY: In rat liver, serine dehydratase mRNA is undetectable in the late prenatal period, but its level increases rapidly after birth to a transient peak, and then after decrease gradually increases again to a maximum 2 weeks after birth that is slightly higher than that of adult liver. To determine whether mature quiescent hepatocytes proliferate without loss of differentiated functions, we measured the serine dehydratase mRNA contents in regenerating liver and primary cultured hepatocytes from adult rats. Partial hepatectomy resulted in a dramatic decrease in the mRNA content within 24 h and then its recovery within a week. In subconfluent cultures of adult rat hepatocytes that did not grow even in the presence of mitogens, serine dehydratase mRNA was maintained at a high level. However, when the hepatocytes were cultured at low cell density without added mitogens, their serine dehydratase mRNA content decreases to a quarter of that of subconfluent cultures. The possibility that the expression of serine dehydratase mRNA is regulated in G0/G1 transition before entry into the S phase and the relationship of the mRNA with growth are discussed.

The liver has a variety of functions involving large numbers of proteins including enzymes, and the contents of these proteins change during development. Neonatal hepatocytes grow actively and are not fully differentiated, but during development, their growth slows down and they express differentiated functions (1). The expressions of various proteins during development can be classified into three groups: early fetal, perinatal and neonatal

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Abbrevations used : MOPS, 3-(N-morpholino) propanesulfonic acid; SDS, sodium dodecyl sulfate; 20 x SSPE, 3 M NaCl, 0.18 M NaH_2PO_4, 20 mM EDTA.

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late suckling clusters, according to the sequential expressions of differentiated markers (2). Serine dehydratase is a liver-specific enzyme and its main physiological role is formation of pyruvate from serine for gluconeogenesis. In rat liver, its activity is negligible in the fetal period, but increases after birth (2-4). Therefore, this enzyme is thought to belong to the perinatal cluster of proteins (2). However, Snell et al. (5,6) found that the activity of this enzyme increases rapidly after birth to an initial transient peak and then to a second peak at weaning. Their results suggest that serine dehydratase must be expressed during terminal differentiation of the liver, like tryptophan 2,3-dioxygenase and glucokinase (2). In contrast to neonatal hepatocytes, those in adult liver are quiescent under normal conditions, but proliferate rapidly after partial hepatectomy. In primary cultures of adult rat hepatocytes, DNA synthesis is induced by the additions of insulin and epidermal growth factor (7-12) or hepatocyte growth factor (13) only at low cell-density (12,14,15). It was also found that in cultures at low cell-density, mature hepatocytes tend to lose their differentiated functions (15).

By RNA blotting hybridization with cDNA of serine dehydratase as a probe, we examined the expression of this enzyme in developing rat liver and in adult rat hepatocytes stimulated to proliferate *in vivo* and *in vitro*. This paper reports that the expression of serine dehydratase mRNA in hepatocytes and their growth seem to be regulated in a reciprocal manner.

MATERIALS AND METHODS

Reagents---[α-^{32}p]dCTP (3000 Ci/mmol) was purchased from New England Nuclear. The Klenow fragment of *E.coli* DNA polymerase I was obtained from Boehringer Mannheim. Restriction endonucleases were from Takara Shuzo, Toyobo, and New England Biolabs.

Treatment of Animals---Wistar strain rats were given laboratory chow *ad libitum*. Fetuses and neonatal rats were obtained from a local supplier of laboratory animals. Fetal ages were assessed, and fetuses were removed by cesarean section under ether anethesia. Neonatal animals were obtained from litters. Male rats weighing 150-200g were subjected to 70 % hepatectomy under ether anethesia (16). The livers of all these animals were used for isolation of total RNA.

Primary Cultures of Hepatocytes---Parenchymal hepatocytes were isolated from adult male Wistar strain rats (150-200 g) by perfusion of the liver *in situ* with collagenase. The isolated cells were cultured as monolayers in Williams medium E with 5 % calf serum as reported previously (17).

RNA Blot Hybridization Analysis---Total RNA was extracted from livers or cultured hepatocytes by the guanidinium thiocyanate method of Chirgwin *et al.* (18). The total RNA (10 μg) was denatured in a solution of 20 mM MOPS buffer (pH 7.0) containing 13 % formaldehyde, 40 % formamide, 5 mM sodium acetate and 1 mM EDTA at 65 °C for 5 min and electrophoresed on 1.0 % agarose gel containing 6 % formamide (19). Then, the RNA was transferred (20) to a Hybond-N nylon membrane (Amersham). The hybridization probe used was a 950 bp DraIII-EcoRV fragment from pSDH4 (21, 22): the probe was labeled with [α-^{32}P]dCTP by the random priming method (23). Before hybridization, the nylon membrane was prehybridized in 50 % formamide, 5 x SSPE, 200 μg/ml sonicated salmon sperm DNA, 0.1% SDS, and 5 x Denhardt's solution at 42 °C for 6 h. Hybridization was performed at 42 °C for 18 h in the same solution containing labeled cDNA (10^6 cpm/ml, 10^9 cpm/μg). After hybridization, the nylon membrane was washed four times with 2 x SSPE containing 0.1 % SDS for 15 min each time at 65 °C and then with 1 x SSPE containing 0.1 % SDS for 30 min at 42 °C. The membrane was autoradiographed with Kodak XAR-5 film at -70 °C with an intensifying screen.

RESULTS AND DISCUSSION

We examined the levels of serine dehydratase mRNA in the livers of rats from day 19 of gestation to various ages after birth. On hybridization of total RNA from fetal liver with the serine dehydratase cDNA probe no 1.5 Kb RNA, corresponding in size to serine dehydratase mRNA (21), could be detected (Fig. 1). After birth, however, the level of this mRNA showed a prompt transient peak and then increased gradually, reaching the adult level 2 weeks after birth. This developmental pattern of change was consistent with the pattern of change in enzyme activity reported by Snell et al.(5,6). As described previously (2,5) the rapid increases in serine dehydratase and other enzymes immediately after birth may be due to an increase in hepatic cyclic AMP concentration resulting from decrease in plasma insulin and rise of plasma glucagon concentration. This possibility is supported by the findings that serine dehydratase in primary cultures of adult rat hepatocytes is induced by the cooperative actions of glucocorticoids and glucagon, but that

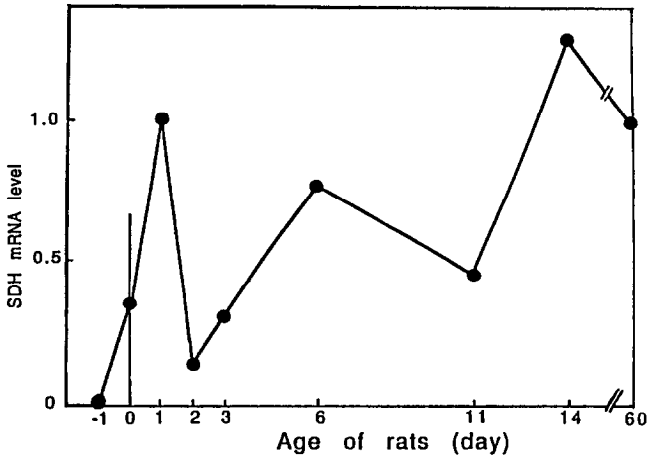

Fig. 1. Changes of serine dehydratase mRNA level in rat liver during development. The level of mRNA for serine dehydratase was measured by densitometric scanning of autoradiograms of hybridized northern blots. Data are shown as ratios to the signal on day 60.

this induction is suppressed by insulin (17,24,25). Thus, the developmental pattern of change in the level of serine dehydratase mRNA is not typical of proteins of the perinatal cluster, but intermediate between those of the perinatal and late suckling clusters. In the perinatal period, hepatocytes are immature and can grow actively (1,26). Previously, in studies on primary cultures of neonatal rat hepatocytes, we found that hepatocytes isolated just after birth grow by an autocrine mechanism, but that this autonomous growth decreases rapidly in a few days after birth (27) and that when hepatocytes acquire the ability to express tryptophan 2,3-dioxygenase, a member of the late sucking cluster, they are no longer able to proliferate (28). The accumulation of serine dehydratase mRNA in hepatocytes apart from that during the transient increase 1 day after birth, seems to be reciprocally correlated with decrease of autonomous growth in neonatal rats.

Next, to determine whether differentiated functions are maintained when adult hepatocytes are stimulated to proliferate, we examined the level of serine dehydratase mRNA in the liver at various times after partial hepatectomy. After partial hepatectomy, the level of the serine dehydratase mRNA decreased

dramatically, being undetectable by autoradiography one day later (Fig. 2). Expression of serine dehydratase mRNA started to increase 3 days after partial hepatectomy and reached the normal level after 7 days. Since the half-life of serine dehydratase mRNA is 8-10 h (25), the transcription of serine dehydratase may be blocked almost completely immediately after partial hepatectomy and then gradually recover after 3 days. But, the alternative possibility that the decrease of this mRNA after partial hepatectomy is due to increase in its turnover is not excluded. Friedman *et al.* (29) examined the expressions of more than ten liver-specific genes during liver regeneration and found that most liver-specific mRNAs did not change greatly in concentration or in transcription rate, but that the levels of several mRNAs, such as the mRNAs of "acute phase" proteins, increased. They concluded that differentiated hepatocytes continue to function as differentiated cells during the one or two replications necessary for regeneration. However, the results in Fig. 2 indicate that some differentiated functions, including expression of serine dehydratase mRNA, must be lost after partial hepatectomy. In addition to increase of mRNAs for "acute phase proteins", a sequence of inductions of growth-related mRNAs in regenerating liver after partial hepatectomy is apparent: namely, transient increases in the levels of mRNAs for c-fos (30 min after partial hepatectomy)

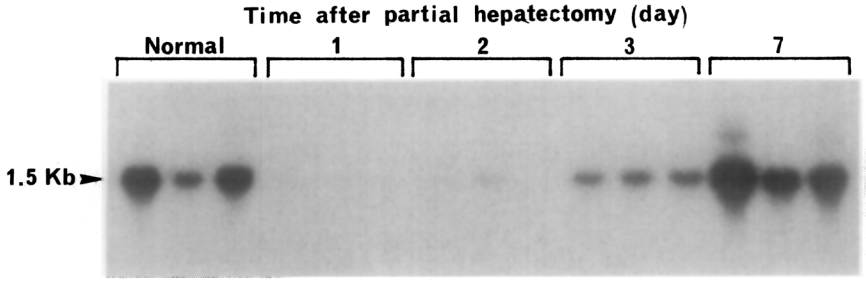

Fig. 2. Decrease of serine dehydratase mRNA after partial hepatectomy. Total RNA was isolated from three rats each before and at the indicated times after partial hepatectomy. Samples of total RNA (10 μg) were analyzed by northern blot hybridization with serine dehydratase cDNA as probe.

(30), c-myc (after 3 h) (31,32), and Ha-ras (after 24 h) (33). The genes encoding actin, tubulin (29), and p53 (34) are activated 8 h after partial hepatectomy, and Ki-ras(35) is activated 12 h after partial hepatectomy. These findings suggest that the genes for serine dehydratase and tryptophan 2,3-dioxygenase and some growth-related genes must be regulated coordinately in a reciprocal manner.

Regenerating liver is a useful system for studies on normal proliferating hepatocytes *in vivo*. But, it is too complex to allow determination of whether the decrease of serine dehydratase mRNA occurs in the G0/G1 transition or during progression towards the S phase. Previously, we demonstrated that in primary cultures of adult rat hepatocytes, the G0/G1 transition is regulated by cell-density: at low cell-density, hepatocytes appear to be in transit from the G0 phase to the G1 phase and respond to the mitogens such as EGF, insulin, and hepatocyte growth factor, but at high cell-density, the G0/G1 transition is suppressed by cell-cell contact (14,15).

Therefore, in this study we cultured adult rat hepatocytes in absence of mitogen at three different cell-densities [1 x 10^5

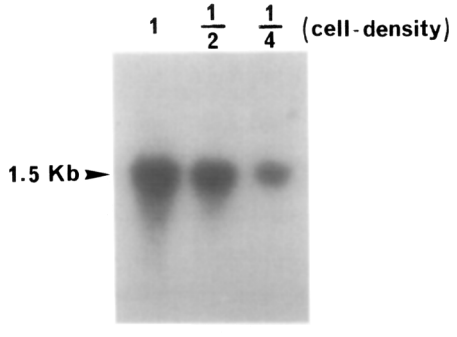

Fig. 3. Cell-density dependency of expression of serine dehydratase mRNA in primary cultures of adult rat hepatocytes. Isolated hepatocytes were cultured at 1 x 10^5 cells/cm^2 (subconfluence, 1), 0.5 x 10^5 cells/cm^2(1/2), and 0.25 x 10^5 cells/cm^2(1/4) in Williams medium E containing 5 % calf serum. Three hours after seeding, the medium was changed to serum- and hormone- free medium. Dexamethasone (10^{-7} M) and glucagon (5 x 10^{-7}M) were added to induce expression of serine dehydratase 20 h after seeding. The cells (about 2 x 10^7 cells) were harvested 6 h after the addition of hormones for measurement of the serine dehydratase mRNA level by northern blot hybridization.

cells/cm^2(subconfluent), 0.5 x 10^5 cells/cm^2, and 0.25 x 10^5 cells/cm^2] and examined their RNA by northern blot hybridization with a serine dehydratase cDNA probe. The level of the mRNA was high in subconfluent cultures and low in cells cultured at low cell-density (Fig. 3). This means that expression of serine dehydratase mRNA decreased during G0/G1 transition. The mechanism of G0/G1 transition and its regulation in hepatocytes are still unknown. Serine dehydratase should be a suitable marker in studies on these problems.

If the decrease in the amount of mRNA of serine dehydratase after partial hepatectomy is caused by regulation of gene transcription, the most interesting point is whether the mechanism of suppression of the serine dehydratase gene expression after partial hepatotectomy is the same as that in fetal liver. Some *trans*-acting transcription factors contribute greatly to cell-specific and tissue-specific gene expressions. We are now studying nuclear proteins from fetal, adult and regenerating livers that can bind 5'-flanking sequence of the serine deydratase gene, by DNase I footprint analysis.

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