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A POSSIBLE ROLE OF C/EBPβ IN TRANSCRIPTIONAL REGULATION OF THE RAT LIVER HAPTOGLOBIN GENE DURING DEVELOPMENT.

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Concerning that the conditions in inter- and extrauterine environment are different, the main role of heterotetrameric glycoprotein haptoglobin (Hp) as hemoglobin-transport protein in the postnatal expression of the Hp is very important especially in the host defense responses to inflammation and infection (Baumann and Gauldie 1994). The systemic response to various forms of injury includes an overexpression of interleukin 1 (IL-1), interleukin 6 (IL-6) and glucocorticoids, alone or in various combinations mediating the induction of the Hp gene mainly in the liver. Elevated level of Hp gene expression leads to a several fold increase of the protein concentration in the circulation of adult rats (Koj *et al.* 1982). In contrast, concentration of Hp in fetal blood is very low (Gitlin and Gitlin 1975) as a consequence of low Hp mRNA level in prenatal liver (Bensi *et al.* 1985). The Hp gene has been classified as one of the genes dominantly expressed after birth (Oliviero *et al.* 1987). However, recent studies showed that a form of Hp aides development of the placenta and fetus (Katnik *et al.* 1995) justifying the activity of Hp gene in fetal liver in response to inflammation. The Hp gene activity in fetal hepatocytes during acute phase is increased to the basal level of Hp expression in uninduced adult liver (Ševaljević *et al.* 1994), indicating the transcriptional activity of the Hp gene in fetal liver also. Both in prenatal and postnatal liver the expression of Hp gene is regulated at transcriptional level, relying essentially on binding affinity of hepatocyte regulatory DNA-binding proteins to the hormone response element (HRE) of the Hp gene (Marinković and Baumann 1990). Since the family of C/EBP proteins cooperates positively with the HRE of the Hp gene (Baumann and Gauldie 1994) and considering that homologous sequence to the consensus transcription factor C/EBPβ-DNA-binding site (Akira *et al.* 1990) in the above gene was espied, we examined whether C/EBPβ transcription factor participates in the transcriptional regulation of the Hp gene in fetal hepatocytes, as well as the time point at which this *trans*-acting protein could be detected during ontogenetic development of the rat liver.

DNA binding affinity of hepatic nucleoproteins was studied by South-Western analysis (Bowen *et al.* 1980). The nucleoproteins from control and turpentine-injected adult or newborn rats as well as the extracts of 19-day-old fetal liver from untreated and inflamed dams were isolated following the procedure elaborated by Gorski *et al.* (1986), separated by SDS-PAGE, blotted and probed with ^{32}P-labelled HRE (-170/-56) of the Hp gene. Sequence specificity of the above interactions was proved in competition experiments in the presence of an excess of unlabelled DNA sequence (data not shown). Comparing to the control patterns (Fig. 1A, lanes 1 and 13), acute phase-induced activation of the Hp gene in fetal liver relies on the induction of newly expressed *trans*-acting proteins with molecular masses of 35, 33, 29 and 20 kD (Fig. 1A, fetus, lane 2), whereas in the adult hepatocytes (Fig. 1A, adult, lane 14) the regulating mechanisms act through enhanced DNA binding affinity of the preexisting protein species (35, 45 and 70 kD). Despite the differences in the regulatory molecular events in fetal and terminally differentiated hepatocytes which are in accordance with the published data (Ševaljević *et al.* 1994), it is important to espy autonomous acute-phase response in prenatal liver implicating the capability of 19-day-old fetal hepatocytes to recognize hormonal mediators involved in the regulation of the Hp gene expression as in adults. Moreover, it was logical outcome that the same trans-acting proteins could express binding affinity to the Hp sequence in the pre- and postnatal liver.

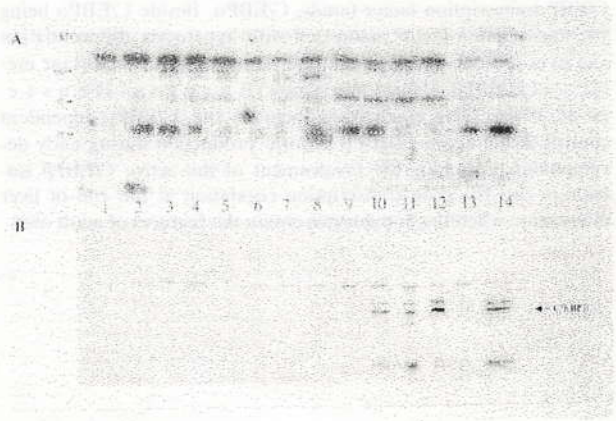

Fig. 1. South-Western (A) and Western (B) blot analysis of hepatic nucleoproteins with hormone response element of the Hp gene (A) and anti-C/EBPβ antibody (B), respectively.
Nucleoproteins are isolated from control (lanes 1, 3, 5, 7, 9) and acute-phase (lanes 2, 4, 6, 8, 10) livers of 19-day-old fetuses (lanes 1, 2), 1-, 3-, 7-, 14-, 21-day-old offspring (lanes 3 - 12) and adult rats (lanes 13, 14). Seven individual livers from a litter were pooled to comprise a single sample. Both analyses were repeated with 3-5 separately isolated nucleoproteins for each time point.

Nucleoprotein with molecular mass of 35 kD (p35) from both, control and acute-phase hepatic patterns of fetal and adult rats, as well as of 1-, 3-, 7-, 14- and 21-day-old offspring (Fig. 1A) displayed binding affinity for HRE of Hp gene. Since the p35 has the same molecular weight as the active isoform of transcriptional factor C/EBPβ, known as liver activatory protein (Descombes and Schibler 1991), following the Western blot procedure (Burnett 1981), the nucleoproteins from control and acute-phase liver of prenatal and postnatal rats were size separated in SDS-PAGE system, transferred onto nitrocellulose sheets and probed using polyclonal rabbit anti-C/EBPβ antibody (Santa Cruz Biotechnology). The C/EBPβ could not be detected in the hepatic extracts from fetal and the first postnatal-week-samples (Fig. 1B, lanes 1 - 6). However, in 14th and 21st day of neonatal

development of both, control and acute phase rat liver, profile of C/EBPβ isoforms corresponded to that observed in the adult pattern (Fig. 1B, lanes 9 - 14) showing remarkable band at 35 kD position.

It should be underlined that in 7-day-old animal we could not detect C/EBPβ in nuclear extract of uninduced liver probably as a consequence of low content of this nucleoprotein (Fig. 1B, lane 7), while the level of C/EBPβ detection in turpentine-treated liver reached the basal one as in control sample of the hepatocytes in 14-day-old newborns (Fig. 1B, lane 8). Our findings pointed that from the end of the first week of postnatal development, 35 kD nucleoprotein displayed the same antigene determinants as C/EBPβ expressed in the adult liver. The obtained differences in DNA-binding affinity of 35 kD-C/EBPβ to the HRE of the Hp gene during acute phase response (Fig. 1A, lanes 7 and 8) may be the result of *de novo* synthesis or posttranslational modification of C/EBPβ (T a k i g u c h i 1998).

It must be outlined that a question about nucleoprotein p35 till the 7th day of postnatal liver development is still open. The answer could be found in the novel role of the other member of C/EBP transcription factor family, C/EBPα. Beside C/EBPα being the transcription factor associated with hepatocyte differentiation and liver-specific development, C/EBPα is also an important mediator of C/EBPβ isoform production (B u r g e s s - B e u s s e *et al.* 1999). This mechanism includes the C/EBPα-dependent control of the active C/EBPβ isoform production during early development presuming the involvement of the active C/EBPβ isoform in the Hp gene transcription regulation at the end of liver maturation when the hepatocytes obtain the features of adult ones.

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