Short communication

Correlation Between Acute Phase-Related Expression of C/EBPβ and Transcriptional Regulation of the Haptoglobin Gene During Rat Liver Development

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Abstract. The hepatic 35 kD nucleoprotein participates in transcriptional regulation of the haptoglobin gene during the development of rat liver. The same molecular mass and *trans*-acting role of the above protein as the active isoform of C/EBPβ transcription factor, made homology between them possible. We could detect C/EBPβ in control hepatic nuclear extracts not earlier than in the second week after the birth. However, the acute-phase reaction induced the expression of 35 kD-C/EBPβ at day 7 of postnatal development suggesting that the above *trans*-active nucleoprotein displays the structural and functional characteristics of C/EBPβ isoform from that day on.

Acute-phase response is a complex reaction of an organism induced by stressful stimulus such as acute inflammation, infection (Koj et al. 1982) or thermal tissue injuries (Ševaljević et al. 1989), followed by remarkable changes in the hepatic protein synthesis and secretion of a specific group of serum proteins known as acute-phase reactants (Baumann 1984). Haptoglobin (Hp) is one of the most prominent acute-phase glycoproteins in the rat displaying pleiotropic activities of which the main one is related to the binding and clearance of hemoglobin (Putnam 1975). Concentrations of this acute-phase reactant in the circulation increase 2.5-fold in adult rat during acute-phase reaction (Koj 1974). In contrast, the concentrations of Hp in fetal blood are very low (Gitlin and Gitlin 1975) as the consequence of the small Hp mRNA amounts in the fetal liver (Bensi et al. 1985). Since the conditions in the inter- and extrauterine environment are different, especially in the view of oxygen distribution, the main role of Hp as hemoglobin-transport protein and the postnatal expression of the Hp gene are obvious. At the same time, recent investigations showed that a form of Hp, known as oncofetal Hp, helps the development of the placenta and the fetus (Katnik et al. 1995). Our previous results showed Hp gene activity in the fetal liver in response to inflammation of the mother to be increased to the basal level of Hp expression in uninduced adult one

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(Ševaljević et al. 1994), indicating the transcriptional activity of the Hp gene in also the fetal liver. Although transcriptional activities of the Hp gene are different in fetal and adult hepatocytes, transcriptional regulation of the Hp gene relies on *cis-trans* interaction between the Hp gene hormone response element (HRE) and nucleoproteins from both livers (De Simone and Cortese 1988). Since the family of C/EBP proteins cooperate positively with HRE of different target genes (Baumann and Gauldie 1994) we investigated the role of C/EBPβ transcription factor in the transcriptional regulation of the Hp gene in fetal and adult hepatocytes as well as the time of occurrence of this *trans*-acting protein during ontogenetic development of the rat liver.

In order to investigate the DNA binding affinities of hepatic nucleoproteins we performed the South-Western analysis as described by Bowen et al. (1980). The nucleoproteins from control and turpentine injected adult or newborn rats as well as extracts of 19-day-old fetal livers from an untreated and inflamed dams were isolated following the procedure developed by Gorski *et al.* (1986), separated by SDS-PAGE, blotted and probed with ^{32}P-labelled HRE (-170/-56) of the Hp gene. The sequence specificity of the above interactions was proved in competition experiments in the presence of an excess of unlabeled DNA sequence (data not shown). Compared to the control patterns (Fig. 1A, lanes 1 and 3), 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 53, 42, 35, 33, 29 and 20 kD (Fig. 1A, lane 2), whereas in the adult hepatocytes (Fig. 1A, lane 4) the regulating mechanisms act through enhanced DNA binding affinity of the preexisting protein species (29, 35, 45, 70 kD). In spite of the differences in the regulator molecular events between fetal and terminally differentiated hepatocytes and our previous results (Ševaljević et al. 1994), it is important to study autonomous acute-phase response in prenatal livers implicating the capability of hepatocytes from 19-day-old fetuses to recognize hormonal mediators involved in the regulation of the Hp gene expression.

At the same time, the nucleoprotein with a molecular mass of 35 kD (p35) from both control and acute-phase hepatic patterns of fetal and adult rats as well as 1-, 3- and 7-day-old offspring (Fig. 1B, lanes 5, 6, 7, 8, 9, 10) had binding affinity for HRE of the Hp gene. Since p35 has the same molecular mass as the active isoform of transcriptional factor C/EBPβ, known as liver activator protein (Descombes and Schibler 1991), following the Western blot procedure (Burnett 1981) the nucleoproteins from control and acute-phase livers of prenatal and postnatal rats were size separated in SDS-PAGE system, transferred onto nitrocellulose sheets and treated with polyclonal rabbit anti-C/EBPβ antibody (Santa Cruz Biotechnology). C/EBPβ could not be detected in the hepatic extracts from fetal and the first postnatal-week-samples (Fig. 2, lanes 1, 2, 3, 4, 5, 6). However, on days 14 and 21 of neonatal development of both, control and acute phase rat livers, the profile of C/EBPβ isoforms corresponded to that observed in the adults (Fig. 2, lanes 9, 10, 11, 12, 13, 14).

It should be stressed that on day 7 after the birth no C/EBPβ could be de-

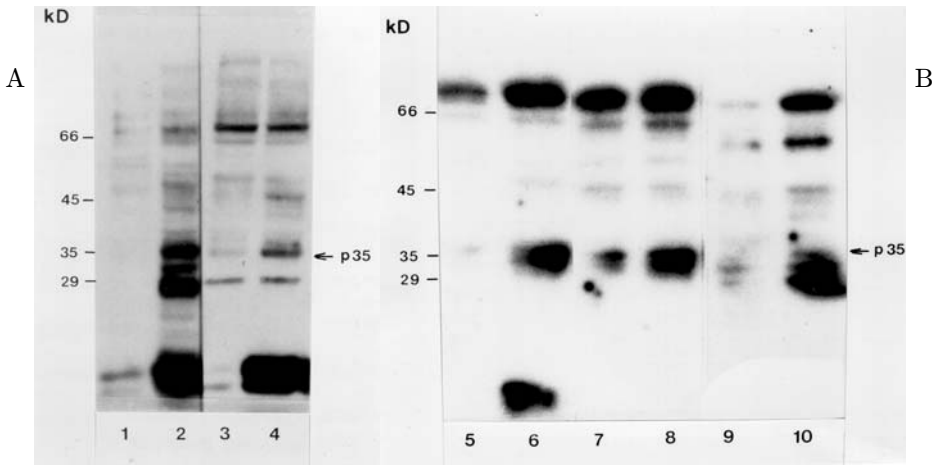

Figure 1. South-Western blot patterns of complexes obtained with the hormone response element of the Hp gene and hepatic nucleoproteins. Nucleoproteins were isolated from control (lanes 1, 3, 5, 7, 9) and acute-phase (lanes 2, 4, 6, 8, 10) livers of 19-day-old fetuses (lanes 1, 2), 1-, 3-, 7-day-old offspring (lanes 5, 6, 7, 8, 9, 10), and adult rats (lanes 3, 4). Seven individual livers from a litter were pooled to comprise a single sample. The South-Western analysis was repeated with 3-5 separately isolated nucleoproteins for each time point.

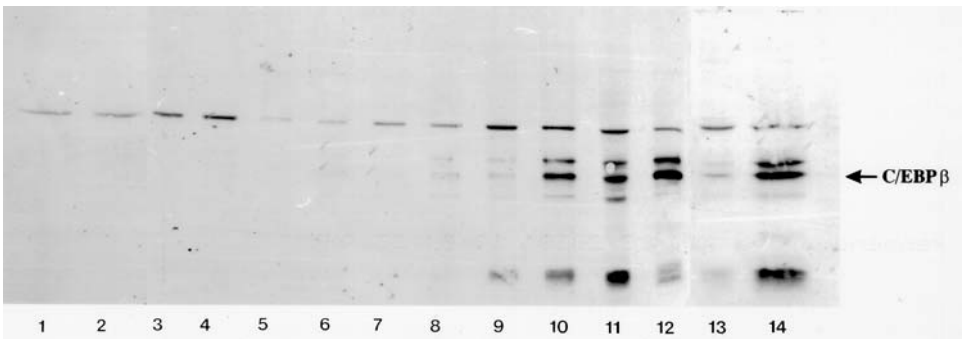

Figure 2. Western blot analysis of hepatic nucleoproteins with anti-C/EBPβ antibody. Nucleoproteins were isolated from control (lanes 1, 3, 5, 7, 9, 11, 13) and acute-phase (lanes 2, 4, 6, 8, 10, 12, 14) livers of 19-day-old fetuses (lanes 1, 2), 1-, 3-, 7-, 14-, 21-day-old offspring (lanes 3, 4, 5, 6, 7, 8, 9, 10, 11, 12), and adult rats (lanes 13, 14). Seven individual livers from a litter were pooled to comprise a single sample. The Western blot analysis was repeated with 3-5 separately isolated nucleoproteins for each time point.

tected in nuclear extracts of noninduced livers, probably because of the low contents of this nucleoprotein (Fig. 2, lane 7), while the levels of C/EBPβ in turpentine-treated livers were the same as control samples of the hepatocytes on day 14 after

the birth (Fig. 2, lane 8). These findings suggested that, from the end of the first week of the postnatal development of rats, the 35 kD nucleoprotein displayed the same antigenic determinants as C/EBPβ in the adult liver. The differences observed in DNA-binding affinity of 35 kD-C/EBPβ to HRE of the Hp gene during acute phase response (Fig. 1, lanes 7 and 8) may be the result of *de novo* synthesis or posttranslational modification of C/EBPβ (Takiguchi 1998).

It should be underlined that the issue of nucleoprotein p35 until day 7 of postnatal liver development remains open. Throughout those development stages, nucleoprotein p35 participates in transcriptional regulation of the Hp gene but not as detectable C/EBPβ isoform. Moreover, C/EBPβ may play a major role in the regulation of the gene expression during the acute-phase reaction but not during the maturation of the liver (Poli 1998) similarly as an other member of the C/EBP transcription factor family, C/EBPα. Besides being the transcription factor associated with hepatocyte differentiation and liver-specific development (Lekstrom-Himes and Xanthopoulos 1998), C/EBPα is also an important mediator of C/EBPβ isoform production (Burgess-Beusse et al. 1999). This mechanism consists of the C/EBPα-dependent control of the active C/EBPβ isoform production during early development. The active C/EBPβ isoform is assumed to be involved in regulation of the Hp gene transcription at the end of liver maturation when the hepatocytes assume the features of adult hepatocytes.

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