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STAT3 AND STAT5b EXPRESSION DURING RAT LIVER DEVELOPMENT AND THE ACUTE-PHASE RESPONSE.

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The signal transducer and activator of transcription (STAT) family of transcription factors are present in a functionally latent, monomeric form in the cytoplasm of resting cells (D a r n e l l, 1997). Following stimulation with cytokines or growth factors and their binding to a cell-surface receptor, STAT factors are rapidly tyrosine-phosphorylated, dimerized, and translocated to the nucleus, where they bind to specific hormone responsive elements of the target genes. There are several members of the STAT family (STAT1, STAT2, STAT3, STAT4, STAT5, and STAT6) with different roles in regulating cell growth, proliferation, and differentiation (M u i, 1999). The liver is a multifunctional organ which emerges from the primitive foregut at day 9 of rat embryonic development (L e m i r e et al., 1991). By the 16th day of gestation, the liver is made up of hepatoblasts (S h i o j i r i et al., 1991), which differentiate into mature hepatocytes. Full liver activity is reached around the third week of postnatal development. Among other functions, the liver plays an important role during the acute-phase (AP) response, a complex systemic reaction of an organism in response to various mechanical, chemical, or biological insults (K o j et al., 1982). As STAT3 and STAT5b were described to be involved in AP response regulation in adults, the aim of this work was to determine their expression throughout rat liver development under normal and AP conditions.

Male and female albino rats of the Wistar strain were used.

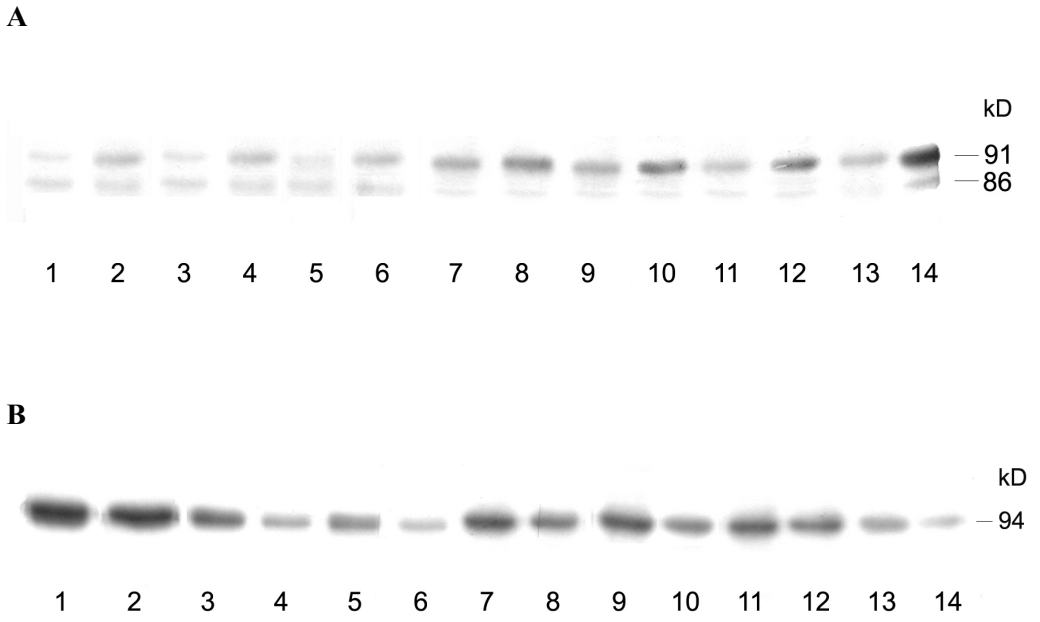

Fig. 1. Immunoblot analysis of rat liver nuclear extracts with STAT3 (A) and STAT5b (B) antibodies. Proteins (20 μg of proteins were separated by 12% SDS-PAGE, electrotransferred onto PVDF membranes, and incubated with polyclonal rabbit antibody raised against rat STAT3 and STAT5b. The antigen-antibody complex was visualized by the ECL detection system. Lanes 1 and 2 – 20-day fetal liver; 3 and 4 – 1st postnatal day; 5 and 6 – 3rd postnatal day; 7 and 8 – 7th postnatal day; 9 and 10 – 14th postnatal day; 11 and 12 – 21st postnatal day; 13 and 14 - 2.5-month-old adult. Lanes 1, 3, 5, 7, 9, 11, and 13 – nuclear proteins isolated from control livers; lanes 2, 4, 6, 8, 10, 12, and 14 – nuclear proteins obtained at 12 h after induction of the AP response.

Livers were isolated from: 20-day-old fetuses removed from 10-week-old dams; 1-, 3-, 7-, 14- and 21-day-old neonatals; and 10-week-old adult males. The livers were from up to five litters of 20-day-old fetuses (i.e., fetuses from up to five dams, depending of the number of the fetuses in each litter) and from 1–, 3-, and 7-day-old neonatals of either the control or the turpentine-treated group. Livers from three neonatals (14- and 21-day-old animals) and three adult (10-week-old) male rats were pooled for each group (control and turpentine-treated). The AP response was induced by a subcutaneous injection of turpentine oil (1 µl/g of body weight) in the lumbar region of the dams, neonatals, and male adults (B a u m a n n et al., 1984). Ether-anesthetized animals were sacrificed 12 h later. Nucleoproteins were isolated from the liver of control and treated rats according to G o r s k i et al. (1986). In order to establish the presence of STAT3 and STAT5b, nucleoproteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (L a e m m l i, 1970) and analyzed by Western immunoblot assay (T o w b i n et al., 1979) using polyclonal STAT3 and STAT5b antibodies (Santa Cruz Biotechnology, USA).

Immunoblot analysis with STAT3 antibody revealed the presence of 91 kD and 86 kD STAT3 isoforms throughout liver development (Fig. 1A). In the fetal liver on the 1st and 7th days of postnatal development, the relative amounts of both isoforms were similar (lanes 1, 3, and 5). From the second postnatal week, the profiles of STAT3 isoforms were similar to those observed in the adult liver, where the amount of the 91 kD isoform was greater than that of the 86 kD STAT3 isoform (Fig. 1A, lanes 7, 9, 11, and 13). During the AP response, the 91 kD STAT3 isoform increased in content, while content of the 86 kD STAT3 was the same as in the corresponding controls (Fig. 1A, lanes 2, 4, 6, 8, 10, 12, and 14). As can be seen in Fig. 1B, STAT5b was detected at all examined time points from the fetal to the adult liver. The relative concentration of STAT5b was considerably higher in the fetal liver (lane 1) compared to samples from postnatal development (lanes 3, 5, 7, 9, and 11) as well as ones from the adult liver (lane 13). It should be noted that induction of the AP response did not affect the relative concentration of STAT5b in the fetal rat liver (lane 2). However, the AP response in the postnatal (lanes 4, 6, 8, 10, and 12) and adult (lane 14) liver was accompanied by decrease in the relative concentrations of STAT5b (Fig. 1B).

Hepatocyte differentiation is still poorly understood because of its complexity and difficulties of manipulation with the embryonic liver. Although in the rat it is initiated around day 9 of embryogenesis, hepatocytes continue to proliferate actively and grow for 2–3 weeks after birth (L e m i r e et al., 1991). Our results revealed that STAT3 is expressed throughout liver development (Fig. 1A), which points to its role during proliferation and differentiation of the rat liver. This information is very important in light of the poorly resolved mechanisms of STAT protein involvement in cellular proliferation and differentiation. To date, it was found that STAT3 is an essential regulatory factor in the early embryonic development of mice (T a k e d a et al., 1997). As for STAT5, two of its isoforms were described, i.e., STAT5a (more prominently expressed in the mammary gland) and STAT5b (the predominant form in the liver) (S t a n d k e et al., 1994; A k i r a et al., 1994). It is known that STAT5 is important in growth stimulation and cell differentiation, which is in good correlation with our results showing the highest level of relative STAT5b concentrations in the fetal liver (Fig. 1B). During acute inflammation, the liver is triggered by pro-inflammatory cytokines, mainly interleukin (IL)-1 and IL-6, which in turn provoke up- or down-activation of transcription factors that regulate different target genes (R u m i n y et al., 2001). Moreover, IL-6-directed transcriptional regulation of target genes in hepatic cells has mostly been correlated with activation of the DNA-binding properties of STAT3, which assumes a principal role in its regulation during the AP response (B o d e et al., 2001; Z h a n g and F u l l e r, 2000). According to the results presented in this study, STAT3 retains its basic role, since relative content of the 91 kD STAT3 isoform (determinated as *trans*-active) was increased during the AP response at all examined time points (Fig. 1A). However, published data suggest that STAT5b also takes part in the transcriptional regulation of a prominent AP protein family member, alpha$_2$-macroglobulin, during the AP response (R i p p e r g e r et al., 1995). That is in correlation with the results presented in this study, where the relative concentrations of STAT5b decreased during postnatal development, but not in the fetal liver (Fig. 1B). R i p p e r g e r et al. (1995) showed that both STAT3 and STAT5b were present in rat liver nuclei during the AP response *in vivo*, suggesting that both regulatory proteins participate in the transcriptional induction of AP genes mediated by IL-6 (to which category the gene for MG belongs).

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