Short Communication

Expression of C/EBPδ in Rat Liver During Development and the Acute-Phase Response

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Abstract. Using Western analysis, C/EBPδ was established in the nuclear extract and nuclear matrix throughout rat liver development and in the adult. During the acute-phase response (APR), C/EBPδ increased in the nuclear extract but remained unchanged in the nuclear matrix of fetal and postnatal rats, whereas it increased in both the nuclear extract and nuclear matrix of the adult. The solubility partitioning of gene regulatory proteins in the nucleus is important for their functioning (Uskoković et al. 2002). The obtained different solubility partitioning profiles of C/EBPδ suggest that its activity is regulated by different mechanisms during development and in the adult.

Key words: C/EBPδ — Nuclear matrix — Nuclear extract — Liver development — APR

CCAAT/enhancer binding proteins (C/EBPs) are a family of basic leucine zipper (bZIP) transcription factors that are involved in the regulation of various aspects of cellular differentiation and functioning. Six different members of the family have been isolated and characterized (C/EBPα to ζ). They all share strong homology in the carboxyl-terminal domain which carries a basic DNA-binding domain and the leucine zipper motif (Poli 1998). C/EBPα is a major regulator of metabolic processes and terminal hepatocyte differentiation during embryogenesis and in the adult (Darlington et al. 1995; Tomizawa et al. 1998). C/EBPβ and C/EBPδ are associated with cell proliferation (Diehl et al. 1995) and the acute-phase response (APR). The APR is a defense reaction of an organism in response to a variety of different insults such as bacterial infection, tissue injury, extensive bleeding or surgery (Ruminy et al. 2001). The liver is supposed to have a very important role in the early stage of the APR. The APR induced by bacterial lipopolysaccharide, turpentine oil, heavy metals or thermal injury results in the repression of C/EBPα expression and a concomitant increase in C/EBPβ and δ mRNA pools in the liver

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(Alam et al. 1992; Gilpin et al. 1996). Increased expression of C/EBPδ was observed during early adipocyte differentiation (Cao et al. 1991).

Hepatocyte differentiation is still poorly understood because of its complexity and difficulties in manipulation with embryonic liver. Although in the rat it is initiated around day 9 of embryogenesis, hepatocytes continue to actively proliferate and grow for 2–3 weeks after birth (Herbst and Babbiss 1990). Since it is not known whether C/EBPδ participates in hepatocyte differentiation, we wanted to determine whether C/EBPδ is expressed throughout rat liver development under normal and acute-phase conditions. In view of the importance of the dynamic spatial (Stenoien et al. 1998) and solubility partitioning (Uskoković et al. 2002) of gene regulators in the nucleus for their appropriate functioning, we examined the partitioning of C/EBPδ between soluble – the nuclear extract, and insoluble – the nuclear matrix, protein fractions. The nuclear matrix is a proteinaceous nuclear substructure that assumes an important role in nuclear functioning (Nakayasu and Berezney 1991), whereas the nuclear extract (Gorski et al. 1986) has been widely used for transcription factor identification and characterization.

Wistar strain albino rats were used. The livers were removed from fetuses (16 and 20 days of gestation), neonatals (1, 7, 14 and 21 days after birth) and adult male rats (2.5 months). The APR was induced by a subcutaneous injection of turpentine oil (1 μl/g of body weight) in the lumbar region of the animals which were subsequently killed at the indicated times. The nuclear matrices and nuclear extracts were isolated from the livers as described by Belgrader et al. (1991) and Gorski et al. (1986), respectively. Proteins were analyzed by SDS-PAGE (Laemmli 1970) and Western immunoblot analysis (Towbin et al. 1979) with a rabbit polyspecific antibody to rat C/EBPδ (Santa Cruz Biotechnology). Immunoreactive bands were identified using an enchanced chemiluminescence (ECL) detection system (Santa Cruz Biotechnology) according to the manufacturer's instructions.

In nuclear matrices isolated from adult livers, C/EBPδ was detected as a 36 kDa protein (Fig. 1A). Analysis of C/EBPδ association with the nuclear matrix at different time points after the induction of the APR revealed that the relative concentration of C/EBPδ increased relative to the control sample. C/EBPδ reached its maximal level 4 h after turpentine treatment, decreased slightly at 6 h, and returned to the control value 12 h after treatment. As in the nuclear matrix, C/EBPδ was detected in the nuclear extract of control adults as 36 kDa protein (Fig. 1A). In accordance with the results obtained with the nuclear matrix, two time points were chosen for analysis of the nuclear extracts isolated from acute phase liver. C/EBPδ peaked at 4 h after treatment and returned to the control level at 12 h.

The observed relative increases in C/EBPδ concentrations in the nuclear matrix and extract during the APR are in agreement with previous reports that C/EBPδ mRNA induction was maximal 3–6 h after the onset of the APR (Rabek et al. 1998). Other authors have detected C/EBPδ mRNA in the adult liver after the induction of the APR, but not C/EBPδ protein in the nuclear extract. Here we demonstrated its presence in the nuclear extract, as well as its association with

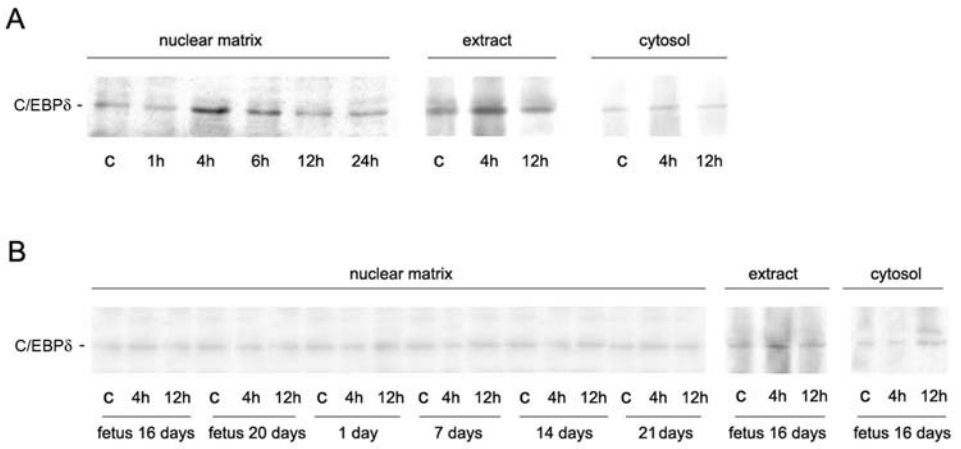

Figure 1. Western analysis of rat liver nuclear and cytosol proteins with anti-C/EBPδ antibody. 20 μg of proteins were subjected to SDS-PAGE, electroblotted onto PVDF membranes and incubated with polyclonal rabbit antibody raised against rat C/EBPδ. Antigen-antibody complex formation was detected using an ECL detection system. **A.** Nuclear matrices, nuclear extracts and cytosols isolated from adult (2.5 months) rat liver. **B**. Nuclear matrices, nuclear extracts and cytosols isolated from rat liver during development. C, control; 1, 4, 6, 12 and 24 h, indicate the times after the induction of the APR.

the nuclear matrix. These findings are consistent with the assumption that newly synthesized C/EBPδ is rapidly transported into the nucleus. Western analysis of the soluble cytoplasmatic protein or cytosol fraction that was obtained after pelleting the nuclei during nuclear extract preparation, revealed the presence of very low concentrations of C/EBPδ (Fig. 1A).

Since C/EBPδ was identified in both nuclear fractions from adult liver, we examined its expression during development. As shown in Fig. 1B, C/EBPδ was detected in the nuclear matrices isolated from fetal and postnatal rat livers. In the control samples, the relative concentrations of C/EBPδ remained unchanged throughout development and were slightly lower than in the adult control nuclear matrix sample (Fig. 1A). The same analysis revealed that after the induction of the APR (4-h and 12-h samples are presented as representative samples) there were no changes in the relative concentrations of C/EBPδ in fetal and neonatal livers. On the basis of these results we concluded that C/EBPδ was expressed in the developing liver. In contrast to the adult nuclear matrix, induction of the APR did not lead to an increase of the concentration of C/EBPδ in the nuclear matrices during development.

To further assess, the expression of C/EBPδ in the developing liver, nuclear extracts isolated from the control and acute phase fetal livers were subjected to

Western analysis. As in the nuclear matrix, C/EBPδ was detected in the nuclear extract from the day 16 of fetal development (Fig. 1B). Its relative concentration was lower than in the nuclear extract prepared from the adult control (Fig. 1A). In contrast to the fetal nuclear matrix, the relative concentration of C/EBPδ in the fetal nuclear extract increased 4 h after the induction of the APR and returned to the control level by the 12^{th} h. As was observed in the nuclear matrix and nuclear extract prepared from adult rats, induction of the APR in the fetus was characterized by an increase in the concentration of C/EBPδ in the nuclear extract relative to the fetal control. C/EBPδ was barely detectable in the fetal cytosols (Fig. 1B) suggesting that the C/EBPδ protein pool was mostly localized in the nucleus.

Our study confirmed that C/EBPδ is expressed in the liver during differentiation. Aside from its presence in the soluble nuclear extract fraction, C/EBPδ was found associated with the insoluble nuclear matrix structure. Previously, we showed that two other members of the C/EBP family, proteins α and β, were preferentially associated with the rat liver nuclear matrix throughout development (Dinić et al. 2000). Literature data indicate that these proteins, especially C/EBPα, are important regulators of hepatic development. However, it is a matter of speculation, if and how C/EBPδ influences hepatic gene expression during development. During the liver regeneration when hepatocyte proliferation is intense, C/EBPα expression was observed to be down-regulated while the expression of C/EBPβ and δ was up-regulated (Diehl et al. 1995). Since C/EBPβ and δ direct adipocyte differentiation (Darlington et al. 1998), it would be interesting to investigate whether C/EBPδ also plays a role in hepatocyte differentiation. The observed differences in C/EBPδ concentrations in the nuclear matrix and nuclear extract during the APR in fetal liver point to the importance of the redistribution of this regulatory protein for its adequate regulation of target genes. Our results also indicate that C/EBPδ solubility partitioning differs in developing and adult rat liver during APR, suggesting that C/EBPδ activity is regulated by different mechanisms.

The described partitioning of C/EBPδ with the soluble and insoluble nuclear protein fractions, the nuclear extract and nuclear matrix respectively, is in agreement with the observation that the localization of transcriptional regulatory proteins in the nucleus is dynamically regulated. Such localization may alter the local concentrations and subsequent assembly of transcription regulatory complexes required for appropriate gene expression. The presented dynamic association of C/EBPδ with the nuclear matrix during development and the APR once again implicates the nuclear matrix in the regulation of transcription. By localizing transcription factors, the nuclear matrix could participate in the establishment of critical transcription factor concentrations near their regulatory sequences.

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References

Alam T., An M. R., Papaconstantinou J. (1992): Differential expression of three C/EBP isoforms in multiple tissues during acute phase response. J. Biol. Chem. **267**, 5021—5024

Belgrader P., Siegel A. J., Berezney R. (1991): A comprehensive study on the isolation and characterization of the HeLa S3 nuclear matrix. J. Cell Sci. **98**, 281—291

Cao Z., Umek R. M., McKnight S. L. (1991): Regulated expression of three C/EBP isoforms during adipose conversion of 3T3-L1 cells. Genes Dev. **5**, 1538—1552

Darlington G. J., Wang N., Hanson R. W. (1995): C/EBPα: a critical regulator of genes governing integrative metabolic processes. Curr. Opin. Genet. Dev. **5**, 565—570

Darlington G. J., Ross S. E., MacDougald O. A. (1998): The role of C/EBP genes in adipocyte differentiation. J. Biol. Chem. **273**, 30057—30060

Diehl A. M., Yang S. Q., Yin M., Lin H. Z., Nelson S., Bagby G. (1995): Tumor necrosis factor-alpha modulates CCAAT/enhancer binding proteins-DNA binding activities and promotes hepatocyte-specific gene expression during liver regeneration. Hepatology **22**, 252—261

Dinić S., Ivanović-Matić S., Bogojević D., Mihailović M., Poznanović G. (2000): C/EBPα and C/EBPβ are persistently associated with the rat liver nuclear matrix throughout development and the acute phase response. Cell Biol. Int. **24**, 691—698

Gilpin D. A., Hsieh C.-C., Kuninger D. T., Herndon D. N., Papaconstantinou J. (1996): Effect of thermal injury on the expression of transcription factors that regulate acute phase response genes: the response of C/EBPα, C/EBPβ, and C/EBPδ to thermal injury. Surgery **119**, 674—683

Gorski K., Carneiro M., Schibler U. (1986): Tissue-specific *in vitro* transcription from the mouse albumin promoter. Cell **47**, 767—776

Herbst R. S., Babbiss L. E. (1990): Regulation of liver gene expression during development and regeneration. In: Mechanisms of Differentiation (Eds. P. B. Fisher and M. Phil), pp. 16—49, C.R.C. Press, Boca Raton, FL

Laemmli U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**, 680—685

Nakayasu H., Berezney R. (1991): Nuclear matrins: Identification of the major nuclear matrix proteins. Proc. Natl. Acad. Sci. U.S.A. **88**, 10312—10316

Poli V. (1998): The role of C/EBP isoforms in the control of inflammatory and native immunity functions. J. Biol. Chem. **273**, 29279—29282

Rabek J. P., Scott S., Hsieh C.-C., Reisner P. D., Papaconstantinou J. (1998): Regulation of LPS-mediated induction of C/EBPδ gene expression in livers of young and aged mice. Biochim. Biophys. Acta **1398**, 137—147

Ruminy P., Gangneux C., Claeyssens S., Scotte M., Daveau M., Salier J.-P. (2001): Gene transcription in hepatocytes during the acute phase of a systemic inflammation: from transcription factors to target genes. Inflamm. Res. **50**, 383—390

Stenoien D., Sharp Z. D., Smith C. L., Mancini M. A. (1998): Functional subnuclear partitioning of transcription factors. J. Cell. Biochem. **70**, 213—221

Tomizawa M., Garfield S., Factor V., Xanthopoulos K. G. (1998): Hepatocytes deficient in CCAAT/enhancer binding protein α (C/EBPα) exhibit both hepatocyte and biliary epithelial cell character. Biochem. Biophys. Res. Commun. **249**, 1—5

Towbin H., Staehelin T., Gordon J. (1979): Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some application. Proc. Natl. Acad. Sci. U.S.A. **76**, 4350—4354

Uskoković A., Vidaković M., Dinić S., Ivanović-Matić S., Martinović V., Poznanović G. (2002): Solubility partitioning of C/EBPβ on the rat hepatocyte nuclear matrix by hydrophobic interactions. Cell Biol. Int. **26**, 451—461

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