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ASSOCIATION OF C/EBPα AND PCNA WITH THE NUCLEAR MATRIX DURING RAT LIVER DEVELOPMENT.

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The nuclear matrix, a residual insoluble proteinaceous nuclear structure, offers a potentially valuable approach for studying nuclear processes. Nuclear matrix proteins are divided into ubiquitously occurring and cell-specific proteins (Korosec *et al.* 1997) such as certain transcription factors (Poznanović *et al.* 1999). It was suggested that by organizing chromatin in a tissue-specific three-dimensional pattern and by localizing transcription factors, the nuclear matrix facilitates interaction of regulatory proteins with their target sites (Getzenberg 1994).

Liver differentiation is a complex process accompanied by morphological changes and coordinated gene expression. In rats, it is initiated at day 9 of embryogenesis and active hepatocyte proliferation and liver growth continue for 2-3 weeks after birth (Herbst and Babiss 1990). It is generally accepted that hepatocyte differentiation is controlled primarily at the level of gene transcription (Zaret 1994). C/EBPα (CCAAT/Enhancer Binding Protein α), a member of the C/EBP family of bZIP transcription factors, is critical for the establishment and maintenance of metabolic processes that are initiated around the 16th day of embryogenesis (Darlington *et al.* 1995), and for the differentiation of hepatocytes from hepatoblasts (Tomizawa *et al.* 1998). One of its functions is to control hepatocyte proliferation *via* the proliferating cell nuclear antigen (PCNA). Expression of PCNA correlates with hepatocyte proliferative activity during differentiation. It decreases significantly at birth and continues to decline over the next 3-4 weeks (Diehl *et al.* 1994). Contrary to PCNA, expression of C/EBPα increases during development and remains high in the adult, as it probably assumes a growth-inhibitory role (Timchenko *et al.* 1997).

Most of the regulatory factors are characterized in the soluble nuclear protein fraction. As we support hypotesis that nuclear matrix might be involved in control and coordination of different nuclear processes during differentiation we examined localization of C/EBPα and PCNA in the nuclear matrix. In order to assess degree of C/EBPα and PCNA binding to the nuclear matrix we compared their relative concentrations in the nuclear matrix with those in transcriptionally active nuclear extract.

Wistar strain albino rats were used. Livers were removed from: (1) fetuses isolated from dams on days 16 and 19 of gestation, (2) 1, 3, 7, 14 and 21-day-old neonates, and (3) 2.5-month-old male adults. The nuclear matrices and nuclear extracts were isolated as described by Belgrader *et al.* (1991) and Gorski *et al.* (1986), respectively. Proteins were analysed by SDS-PAGE (Laemmli 1970) and Western immunoblotting (Towbin *et al.* 1979) using polyspecific antibody to rat C/EBPα (Santa Cruz Biotechnology) and monoclonal antibody to PCNA (DAKO).

Throughout development and in the adult the nuclear matrices contained multiple C/EBPα isoforms of 30, 35, 38, 42 and 45 kDa (Fig. 1A). It was proposed that C/EBPα isoforms are formed by alternative translational initiation from different AUG codons of a single mRNA (Calkhoven *et al.* 1994). In fetal liver, relative concentrations of the isoforms remained unchanged (lanes 1-2). Birth was accompanied by a considerable increase in the concentration of the 30, 38 and 45 kDa isoforms (lane 3) which was maintained during the postnatal period of the liver differentiation (lanes 4-7). In the adult, relative concentrations of all nuclear matrix-associated isoforms increased further, most notably the 30, 42 and 45 kDa isoforms (lane 8).

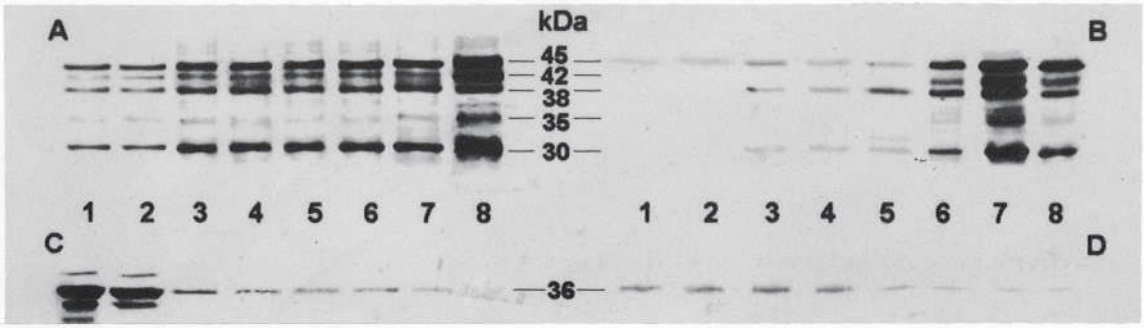

Fig. 1. Western analysis of rat liver nuclear matrix (A, C) and nuclear extract (B, D) proteins with anti-C/EBPα antibody (A, B) and anti-PCNA antibody (C, D). Twenty μg of nuclear proteins were subjected to Western analysis as described. Antigen-antibody complex formation was detected using BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium). Lane 1 - 16 days fetal liver, 2 - 19 days, 3 - 1st postnatal day, 4 - 3rd day, 5 - one week, 6 - two weeks, 7 - three weeks, 8 - 2.5-month-old adult. Bars indicate the molecular masses of the C/EBPα isoforms and PCNA.

Compared to the nuclear matrix, relative concentrations of C/EBPα isoforms in the adequate nuclear extracts were significantly lower (Fig. 1B). In nuclear extracts from fetal liver, only the 45 kDa isoform was detected (lanes 1-2). On days 1, 3 and 7 after birth, C/EBPα was represented by the 30, 38 and 45 kDa isoforms (lanes 3-5). By day 14, all five isoforms were detected in the nuclear extract (lane 6). Maximal concentrations of all isoforms were observed on the 21st postnatal day (lane 7) when their relative concentrations were higher than in the respective nuclear matrix pattern, and in the adult nuclear extract (lane 8). The observed overall augmentation of C/EBPα in the nuclear matrix is in agreement with detected increase in concentrations and number of C/EBPα isoforms in the nuclear extract during development and postnatally. Established preferential association of C/EBPα with the nuclear matrix in differentiated liver, as well as during development, could reflect the importance of interactions of C/EBPα with the nuclear matrix for its *in vivo* functioning.

As shown in Fig. 1C, relative concentrations of PCNA in the nuclear matrices were the highest during embryogenesis (lanes 1-2). After birth, relative concentration of nuclear matrix-associated PCNA decreased until the third postnatal week (lanes 3-7). In the adult nuclear matrix, PCNA was at the limit of detection (lane 8). Similarly, relative concentrations of PCNA were the highest in nuclear extracts from fetal livers (Fig. 1D, lanes 1-2) and in neonatal liver on 1st and 3rd day after birth (lanes 3 and 4). In subsequent days of postnatal development, relative PCNA concentration decreased (lanes 5-7) and in the adult nuclear extracts PCNA was barely detectable (lane 8).

The alternating nuclear matrix and extract concentrations of C/EBPα and PCNA are in agreement with the proposed model on the role these proteins play in regulating hepatocyte growth. Briefly, in quiescent hepatocytes, high levels of C/EBPα maintain a p21 protein level which binds PCNA and inhibits PCNA-dependent DNA replication. When C/EBPα is reduced, p21 is also decreased and permits an increase in PCNA activity and hepatocyte proliferation rate (T i m c h e ń k o *et al.* 1997).

Partitioning of C/EBPα and PCNA in both nuclear protein fractions, matrix and extract, could suggest that the regulatory activity of C/EBPα and PCNA is influenced by their ability to interact with both nuclear compartments. The data documenting specific interactions of nuclear matrix proteins with DNA (G e t z e n b e r g 1994), and here presented dynamic associations of C/EBPα and PCNA with the nuclear matrix during liver development, further support the proposed mechanism that nuclear matrix could facilitate gene expression by concentrating and localizing regulatory proteins near their target sites.

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