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# DYNAMIC ASSOCIATIONS OF TRANSCRIPTION FACTORS WITH THE RAT LIVER NUCLEAR MATRIX ARE FUNCTIONALLY RELATED TO DIFFERENTIAL ALPHA-2-MACROGLOBULIN GENE EXPRESSION

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*Abstract* — Participation of the nuclear matrix in regulation of alpha-2-macroglobulin (α2M) gene transcription during rat liver development and the acute-phase (AP) response are examined. DNA affinity chromatography of fetal and adult liver internal nuclear matrix proteins under basal and AP conditions with the α2M gene promoter (-852/+12) and immunoblot analysis revealed diverse patterns of association of transcription factors with the nuclear matrix. HNF-6, C/EBPα, and STAT5b were involved in basal and C/EBPβ, STAT1, and STAT3 in AP-stimulated α2M expression. These findings support the assumption that transcription factor-nuclear matrix interactions serve to channel gene regulatory proteins to DNA sequences.

*Key words:* Alpha-2-macroglobulin, acute phase response, rat liver, nuclear matrix, transcription factors

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## INTRODUCTION

Aspects of the spatial and temporal organization of the genome and factors that regulate its activity exert an important influence on its functioning (Misteli, 2005). The activity of genes depends on the three-dimensional context in the nucleus, which is related to interactions between DNA elements referred to as S/MARs (Scaffold/Matrix Attachment Region) (Mirkovitch et al., 1984) and the nuclear matrix, a three-dimensional network of non-histone proteins (Berezney et al., 1995). By anchoring S/MARs, the nuclear matrix is involved in chromatin loop formation (Laemmli et al., 1992). Chromatin loops represent independent units of replication and transcription that are isolated from the regulatory effects of neighboring loops (Bonifer et al., 1991). Aside from being architectural DNA elements, S/MARs also possess enhancer activity (Sjakste, 1993). They are positioned near gene regulatory elements and many transcription factor-binding sites (Boulikas, 1994). The interaction of S/MARs with the nuclear matrix suggests that gene localization in the nucleus is closely related to transcriptional activity (Sjakste and Sjakste, 2001; Heng et al., 2004). The association of multienzyme complexes such as SWI/SNF (switch/sucrose non-fermenting) that use ATP hydrolysis to mobilize nucleosomes (Reyes et al., 1997), histone acetyl transferases (HATs) that modify histones in the nucleosome core (Sterner and Berger, 2000), and histone deacetylases (HDACs) (de Ruijter et al., 2003) with the nuclear matrix (Hendzel et al., 1994) points to involvement of the nuclear matrix in changes of chromatin organization during transcriptional activation.

Different *trans*-acting regulatory proteins have been found to be associated with the nuclear matrix (van Wijnen et al., 1993; Bidwell et al., 1993; Guo et al., 1995; Nardozza et al., 1996; Lindenmuth et al., 1997; Stenoien et al., 2001). The discovery of nuclear matrix targeting signals (NMTS) in certain tissue-specific *trans*-activators (De Franco and Guerrero, 2000) and the identification of nuclear matrix proteins

that recognize them (Oesterreich et al., 2000; Townson et al., 2003) provide additional evidence that nuclear matrix proteins establish direct interactions with regulatory proteins. We previously identified dynamic associations between members of the C/EBP family of transcription factors and the rat liver nuclear matrix during development (Dinić et al., 2000, 2004, 2005; Ivanović–Matić et al., 2000) and after induction of the acute-phase (AP) response, an early systemic reaction to different traumas in the adult (Kushner, 1982; Bogojević et al., 2003; Poznanović et al., 1999). We proposed that gene regulation of IL-6-type-cytokine-responsive genes, and probably gene regulation in general, is an integrative process in which the activities of several transcription factors are fine-tuned by local changes in the nuclear microenvironment through the agency of the nuclear matrix (Mihailović et al., 2007; Uskoković et al., 2002; 2007).

Rat alpha-2-macroglobulin ($\alpha_2$M) is a plasma glycoprotein which is synthesized in the liver and involved in different aspects of development and the AP response. As a part of the complex defense mechanism activated by inflammation, the AP response serves to reestablish physiological homeostasis after its disturbance by either chemical or mechanical insults, bacterial or viral infections, or neoplasia. During the AP response, $\alpha_2$M gene expression is increased in the fetus 2-fold and in the adult 5-fold (Glibetić et al., 1992). Under basal physiological conditions, $\alpha_2$M gene expression is high in the fetal liver, but extremely low in the adult. An IL-6 responsive element (RE) was identified in the $\alpha_2$M gene (Northemann et al., 1988; Kunz et al., 1989), and binding of the cytokine-inducible transcription factors STAT1 and STAT3, the early regulators of transcription, has been shown (Wegenka et al., 1994). In addition, several potential C/EBP binding sites were characterized in the $\alpha_2$M gene promoter region (Baumann et al., 1992; Bogojević et al., 2003). STAT5b also displays binding affinity toward the IL-6 RE of the rat $\alpha_2$M gene. It participates in the gene's transcription under basal conditions, as well as in its induction during the AP response (Ripperger et al., 1995). Despite the accumulated data, details concerning the regulation of $\alpha_2$M gene transcription during liver development have yet to be established.

In this work, associations of HNF-6, C/EBPα, C/EBPβ, STAT 1, STAT3, and STAT5b with the rat liver nuclear matrix isolated from fetal and adult livers under basal and AP conditions were analyzed by immunoblot analysis. Nuclear matrix-associated transcription factors were considered to be involved in transcription if they displayed binding affinity toward the extended $\alpha_2$M gene promoter (-852/+12) under conditions of DNA affinity chromatography. The DNA-bound species of nuclear matrix-associated transcription factors were subsequently identified by immunoblot analysis. The associations of transcription factors with the nuclear matrix and their DNA binding profiles were dynamic, correlating with differential $\alpha_2$M gene transcription. The presented results suggest that the nuclear matrix regulates transcriptional activity by concentrating regulatory proteins and directing them to their target sites.

## MATERIALS AND METHODS

### *Animals*

All the animal procedures were approved by the Committee for Ethical Animal Care and Use of the Institute for Biological Research, Belgrade, which acts in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institute of Health (NIH Publication No. 85/23, revised in 1986). Male and female albino rats of the Wistar strain (*Rattus norvegicus*) were used. Livers were isolated from: 20-day-old fetuses removed from 2.5-month-old dams; and 2.5-month-old male adults. To obtain sufficient material for one experiment, the livers were pooled from up to five litters of 20-day-old fetuses (i.e., fetuses from up to five dams, depending on the number of fetuses in each litter), either from the control or from the turpentine-treated groups. Livers from three adult (2.5-month-old) male rats were pooled in each group (control and turpentine-treated) for one experiment. Serums were prepared from 20-day-old fetuses, neonatal (1-, 7-, and 21-day-old) and adult (10-week-old) male rats. For every analyzed stage

of development, the serum sample was obtained by pooling the blood: from three litters in the case of 20-day-old fetuses, 1- and 7-day-old neonatal rats, and three 21-day-old and adult animals. For each group of rats, 3-5 separate experiments were performed. The AP response was induced by a subcutaneous injection of turpentine oil (1 μl/g of body weight) to the lumbar region of the dams, neonatal, and male adult rats (Baumann et al., 1984). The animals were killed 12 h after turpentine injection. The rats were kept in conditions of constant temperature, humidity, and light (12 h of light and 12 h of darkness).

*Measurement of serum $\alpha_2$M concentrations*

The concentration of $\alpha_2$M was determined by rocket immunoelectrophoresis with anti-human $\alpha_2$M antibody (Sigma-Aldrich). The method (Laurell, 1972) is based on the immunoprecipitation reaction between the antigen ($\alpha_2$M) present in the serum and the monospecific antibody (anti-$\alpha_2$M) incorporated in the agarose gel at points of their optimal concentration, which are characterized by the formation of immunoprecipitation peaks (so-called "rockets"). The concentration of the antigen in the serum (sample) is proportional to the area under the formed precipitation peak. The area is used to quantify the antigen in the serum (determine its relative concentration). Absolute concentrations were not measured. Every "rocket" (corresponding to the control and indicated times after turpentine injection) was drawn out on tracing paper and their areas established (integrated). The values obtained after quantification were expressed as the means +/- SEM from three separate experiments, according to Hoel (1966).

*Rat liver nuclear matrix preparation*

Rat liver nuclei were isolated and purified according to Kaufmann and Shaper (1984). All steps were carried out at 4°C, unless otherwise indicated. All buffers contained 1 mM phenylmethylsulfonyl fluoride (PMSF). Rat livers (0.5 g/ml) were minced in STM buffer (0.25 M sucrose, 50 mM Tris-HCl, pH 7.4, and 5 mM $MgSO_4$) and homogenized with several strokes in a Potter-Elvehjem Teflon-glass homogenizer. The homogenates were centrifuged at 1,000xg for 15 min in a Sorvall SS-34 rotor and washed once with STM buffer. The crude nuclear pellet was resuspended in DS buffer (2.2 M sucrose, 50 mM Tris-HCl, pH 7.4, and 5 mM $MgSO_4$), layered over a 5 ml cushion of the same buffer, and centrifuged at 72,000xg for 60 min in a Beckman SW-28 rotor. The pellet was resuspended in STM buffer, layered over a cushion of DS buffer, and centrifuged at 72,000xg for 30 min. The nuclear matrix was isolated from the purified nuclei by the procedure of Belgrader et al. (1991) with certain modifications. Nuclei were stabilized by incubating at 42°C for 20 min, incubated with 2 mM sodium-tetrathionate in STM buffer for 1 h, and then washed twice with the same buffer without sodium tetrathionate by centrifugation at 5,000xg for 10 min. The nuclei were resuspended in STM buffer ($10^8$ nuclei/ml), incubated with 100 μg/ml DNase I for 1 h at 30°C, and subjected to consecutive extraction/centrifugation steps: twice with high-salt buffer (0.25 M $(NH_4)_2SO_4$, 10 mM Tris-HCl, pH 7.4, and 0.2 mM $MgSO_4$), once with freshly prepared 1% Triton X-100 in low-salt buffer (10 mM Tris-HCl, pH 7.4, and 0.2 mM $MgSO_4$), and washed twice with low-salt buffer. Centrifugations were carried out at 8,000xg for 15 min. Aliquots of nuclear matrices were resuspended in 0.25 M sucrose, 10 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.4, and 0.5 mM PMSF to which an equal volume of sterile glycerol was added, then kept at -20°C.

*Preparation of internal nuclear matrix*

As only proteins soluble under near physiological conditions can be analyzed by DNA affinity chromatography, a soluble protein fraction of the isolated nuclear matrix, the internal nuclear matrix (INM), was isolated. Preparation of INM proteins was carried out as described by Stuurmann et al. (1990). The nuclear matrix was first resuspended in low-salt buffer ($2 \times 10^8$/ml). An equal volume of 2 M NaCl and 40 mM DTT in the same buffer was added to the suspension. After incubation for 20 min on ice, insoluble nuclear matrix proteins were removed by centrifugation at 10,000xg for 15 min. The supernatant was dialyzed against 10 mM ammonium acetate (pH 7.4) with several changes of buffer and

stored lyophilized at -70°C.

*SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analysis*

Protein concentrations were determined according to Lowry et al. (1951). For SDS-PAGE, proteins (20 μg) were loaded onto 4% stacking/12% separating slab gels as described by Laemmli (1970). Proteins separated by SDS-PAGE were transferred to PVDF membranes (Hybond-P, Amersham Pharmacia Biotech), and immunoblot analysis was performed by the procedure of Towbin et al. (1979) using polyclonal antibodies for rat transcription factors: HNF-6, C/EBPα, C/EBPβ, STAT1, STAT3, and STAT5b (Santa Cruz Biotechnology). After incubation with blocking solution (0.05% Tween 20, 50 mM Tris-HCl, pH 7.6, 150 mM NaCl, and 3% non-fat milk), the membranes were incubated with specific antibodies for 2 h at room temperature. After rinsing, the blots were incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulin as a secondary antibody for 1 h. Immunoreactive bands were identified by the enhanced chemiluminescence (ECL) detection system (Santa Cruz Biotechnology) according to the manufacturer's instructions. Western immunoblots were quantified using TotalLab (Phoretix) electrophoresis software (ver. 1.10), and changes in the relative concentrations of transcription factors in different protein fractions were compared.

*DNA affinity chromatography*

Affinity chromatography of sequence-specific DNA binding proteins from rat liver was performed by a slightly modified version of the method of Kadonaga and Tjian (1986). The extended promoter of the male Wistar rat $\alpha_2$M gene (-852/+12, obtained from Dr. Peter Heinrich, Institute für Biochemie an der RWTH Aachen, Aachen, Germany), was annealed and ligated to obtain oligomers and then covalently coupled to Sepharose CL-2B with cyanogen bromide to yield the affinity resin. The DNA affinity resin was equilibrated in a Bio-Rad Econo-Column with dialysis buffer (25 mM Hepes pH 7.6, 0.1 mM EDTA, 1 mM DTT, and 10% glycerol) containing 0.1 M KCl. Equal quantities of internal nuclear matrix proteins prepared from control and treated animals were combined with competitor DNA (salmon sperm) and allowed to stand for 30 min, then passed through the DNA-Sepharose resin by gravity flow. The resin was washed with dialysis buffer containing 0.1 M KCl. The passage of buffer through the column was stopped, and dialysis buffer containing 1 M KCl was added to the column. The resin was mixed with the buffer using a glass rod and allowed to stand for 10 min. The eluate was collected after passing the buffer through the column.

Identification of putative transcription factor binding sites in the extended $\alpha_2$M gene promoter element (-852/+12) was performed by computer search using Alggen Promo software, available on web page: www.alggen.lsi.upc.es (Farré et al., 2003).

*Computer analysis for potential MAR elements and transcription factor binding sites*

MAR-Finder available on web page: www.futuresoft.org/modules/MarFinder/index.html and WebSIDD [Stress Induced (DNA) Duplex Destabilization] available on web page: www.genomecenter.ucdavis.edu/benham/sidd (Bi and Benham, 2004) algorithms were used to search for potential MAR. For that purpose, the 5' region of the $\alpha_2$M gene (-4528/+3235) containing the analyzed promoter region (-852/+12), two introns, and three exons (Northemann et al., 1988) was analyzed. The sequence for analysis was obtained from the gene bank database (NCBI Sequence Viewer, M 23567). It contains 4528 bp upsTream from the coding region (-4528/-1); exon (Ex) 1, which is 167 bp long (+1/+167); intron (In) 1, which is 1871 bp long (+168/+2038); Ex 2 which is 184 bp long (+2039/+2222); In 2, which is 775 bp long (+2223/+2997); Ex 3, which is 157 bp long (+2998/+3154); and part of In 3, 81 bp long (+3155/+3235).

## RESULTS

Involvement of the nuclear matrix in mediation of the availability of gene regulatory proteins was studied by investigating associations of activated transcription factors with the nuclear matrix.

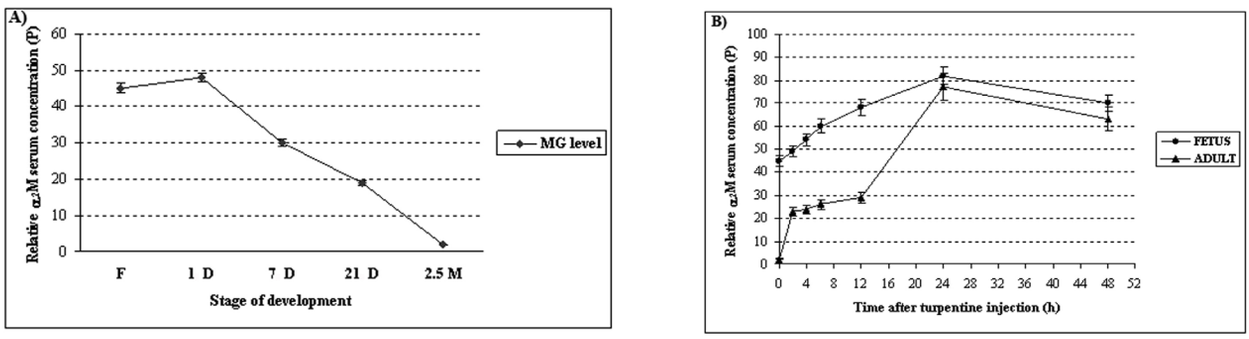


**Fig. 1.** Changes of the relative concentration of α2M in the serum during development (A) and in the course of the AP response induced by turpentine injection (B). The relative concentrations of α2M in the serum were determined by rocket immunoelectrophoresis with anti-α2M antibody as described in Materials and Methods.

Proteins involved in *trans*-regulation of the $\alpha_2$M gene were selected, given that its expression in the rat liver undergoes distinct dynamic changes in response to different stimuli (Fletcher et al., 1988; Kunz et al., 1989; Glibetić et al., 1992). Examination of the concentration of $\alpha_2$M in the serum during development revealed that its expression was highest in the fetal and neonatal stages of growth (Fig. 1A). It peaks on the 20$^{th}$ day of development, then postnatally gradually decreases and in the adult stabilizes at a very low level. Thus, in the 20-day-old fetus the relative serum concentration of $\alpha_2$M was about 22 times higher than in the adult. Another important feature of $\alpha_2$M expression is its permissiveness to induction by molecular signals that are incorporated in the AP response (Kunz et al., 1989). Examination of the serum concentration of $\alpha_2$M after induction of the AP response by turpentine injection (Fig. 1B) revealed its gradual increase in the 20-day-old fetus and a very rapid rise in the adult. Hence, at 24 h of the AP response in both the fetus and the adult, effectively the same absolute maximal concentration of $\alpha_2$M is reached. In the fetus, the increase represents the outcome of a nearly two-fold change of $\alpha_2$M concentration from the basal level, whereas in the adult it results from a dramatic 40-fold change. Subsequently, at 48 h after initiation of the AP response, the concentration of $\alpha_2$M in circulation starts to decrease. The presented results are consistent with the documented profile of $\alpha_2$M expression during the AP response (Ševaljević et al., 1989). It reaches its maximal concentration in circulation at 24 h as the consequence of elevated transcription of the $\alpha_2$M gene established at 12-14 h and results in the generation of maximal mRNA levels at about 18 h after turpentine administration (Northemann et al., 1985; Birch and Schreiber, 1986).

In the adult rat, $\alpha_2$M gene transcription is induced by IL-6 and regulated by STAT3 and STAT5b. These signals are incorporated in the systemic signaling network that gives rise to the AP reaction (Ripperger et al., 1995). However, in the fetal liver, regulation of $\alpha_2$M gene transcription remains partly unresolved. Using the ALGGEN PROMO algorithm for predicting consensus protein binding sequences on the extended $\alpha_2$M gene promoter element (-852/+12) (Fig. 2), we identified putative binding of HNF-6, C/EBP proteins α and β, and STAT proteins 1, 3, and 5b. We then examined whether the patterns of interactions between members belonging to the HNF, C/EBP, and STAT families of transcription factors and the nuclear matrix bore functional significance to the state of $\alpha_2$M gene expression in the 20-day-old fetus, in the 2.5-month-old adult, and during maximal transcriptional activity of the $\alpha_2$M gene at 12 h after induction of the AP response. Changes in $\alpha_2$M gene promoter-protein binding activity of nuclear matrix-associated proteins, that is, the presence of promoter-binding proteins associated with the nuclear matrix, were assessed by DNA affinity chromatography of solubilized internal nuclear matrix proteins with the extended $\alpha_2$M gene promoter element (-852/+12) and subsequent identification of the bound proteins by immunoblot analysis.

-- PROMO predictions detail ----------
Factor name; Start position; End position; Dissimilarity; String; RE equally; RE query
HNF-6alpha[T03257];-460;-448;7.060301; TAAAGTCAATACC; 0.00306; 0.00494;
C/EBPalpha [T00104]; -847; -842; 0.438494; GCAACG; 0.85449; 0.80220;
C/EBPalpha [T00104]; -816; -811; 1.287684; AATTGC; 0.85449; 0.91942;
C/EBPalpha [T00104]; -775; -770; 1.259886; GCAAAA; 0.85449; 0.91942;
C/EBPalpha [T00104]; -751; -746; 1.698380; GCAAAG; 0.85449; 0.91942;
C/EBPalpha [T00104]; -587; -582; 2.958266; GCAAGC; 0.64087; 0.55738;
C/EBPalpha [T00104]; -361; -356; 2.958266; GCTTGC; 0.64087; 0.55738;
C/EBPalpha [T00104]; -296; -291; 2.958266; CCTTGC; 0.64087; 0.55738;
C/EBPalpha [T00104]; -47; -42; 1.698380; GTTTGC; 0.85449; 0.91942;
C/EBPbeta [T00017]; -819; -812; 9.849989; TGAAATTG; 0.13351; .18033;
C/EBPbeta [T00017]; -693; -686; 3.723364; ACTTTTCA; 0.01335; .01874;
C/EBPbeta [T00017]; -673; -666; 9.709372; AGAAATGA; 0.18692; 0.21908;
C/EBPbeta [T00017]; -621; 614; 14.239254; AGAAATTC; 0.16022; 0.20270;
C/EBPbeta [T00017]; -528; -521; 6.656505; GGAAACCT; 0.08011; .09543;
C/EBPbeta [T00017]; -518; -511; 9.709372; TCATTTCT; 0.18692; .21908;
C/EBPbeta [T00017]; -494; -487; 8.919148; CATTTTCA; 0.13351; .17705;
C/EBPbeta [T00017]; -425; -418; 7.057466; TGCTTTCC; 0.13351; .14354;
C/EBPbeta [T00017]; -226; -219; 10.379869; AAGTTTCA; 0.14687; 0.16951;
C/EBPbeta [T00017]; -208; -201; 13.930507; AGAAAAAG; 0.16022; 0.20270;
C/EBPbeta [T00017]; -188; -181; 7.723367; GGAAAGTC; 0.12016; 0.14759;
C/EBPbeta [T00017]; -111; -104; 12.068825; CTCTTTCA; 0.12016; 0.15176;
STAT1 [T01575]; -108; -99; 5.537451; TTTCAGAGAA; 0.03505; 0.04577;
STAT3 [T01493]; -173; -162; 13.050354; CTTCTGGGAATT; 0.01752; 0.01639;
STAT5A [T04683]; -176; -164; 2.999748; ATCCTTCTGGGAA; 0.00188; 0.00253;
STAT5B [T04761]; -683; -672; 10.741335; TTCTTAAAACAG; 0.02253; 0.03121;
STAT5B [T04761]; -172; -161; 4.895795; TTCTGGGAATTC; 0.00501; 0.00677;
STAT5B [T04761]; -110; -99; 9.937531; TCTTTCAGAGAA; 0.01293; 0.01814;

**Fig. 2.** Summary of computer-assisted search for the putative binding sites of selected transcription factors (HNF-6, C/EBPα, C/EBPβ, STAT1, STAT3 and STAT5b) in the extended α2M gene promoter element (-852/+12) using Alggen Promo software, V 3.0.2. (http://alggen.lsi.upc.es). Factor name – transcription factor with its TRANSFAC (V 8.3) database accession number; Start and End – start and end positions of putative binding sequences, respectively; Dissimilarity – rate of dissimilarity (%) between the putative and consensus sequences; String – nucleotide sequence of potential binding site; Random Expectation (RE) – expected occurrences of the match in a random sequence of the same length as the query sequence according to the dissimilarity index (RE equally - equiprobability for the four nucleotides and RE query – nucleotide frequencies as in the query sequence).

Immunoblot analysis of total nuclear matrices (Fig. 3), regardless of whether they were prepared from control (lanes 1) or AP (lanes 4) fetuses or from control (lanes 7) or AP (lanes 10) adults revealed the presence of complete sets of HNF-6 (51 and 55 kD), C/EBPα (45, 42, 38, 35 and 30 kD), C/EBPβ (38, 35, and 30 kD), STAT1 (91 and 84 kD), STAT3 (91 and 86 kD), and STAT5b (94 kD). However, the profiles of nuclear matrix-associated transcription factors that have binding activities for the $\alpha_2$M gene promoter showed subtle differences. During basal transcription, nuclear matrices prepared from the 20-day-old fetus (lanes 3) and the adult (lanes 9) exhibited HNF-6 (55 and 51 kD isomers), C/EBPα (45 and 30 kD isomers), C/EBPβ (35 kD isomer), STAT1 (91 kD), and STAT5b (94 kD) binding activities for the $\alpha_2$M gene promoter. In contrast to the fetus, the nuclear matrix isolated from the adult liver had a DNA binding activity that corresponded to the 84 kD STAT1 isoform. Stimulation of $\alpha_2$M gene transcription after AP induction in both the fetus (Fig. 3, lanes 6) and the adult (lanes 12) was accompanied by similar qualitative and quantitative changes in promoter-protein-binding profiles compared to the basal state. They were characterized by disappearance of the 45 kDa C/EBPα isoform bind-

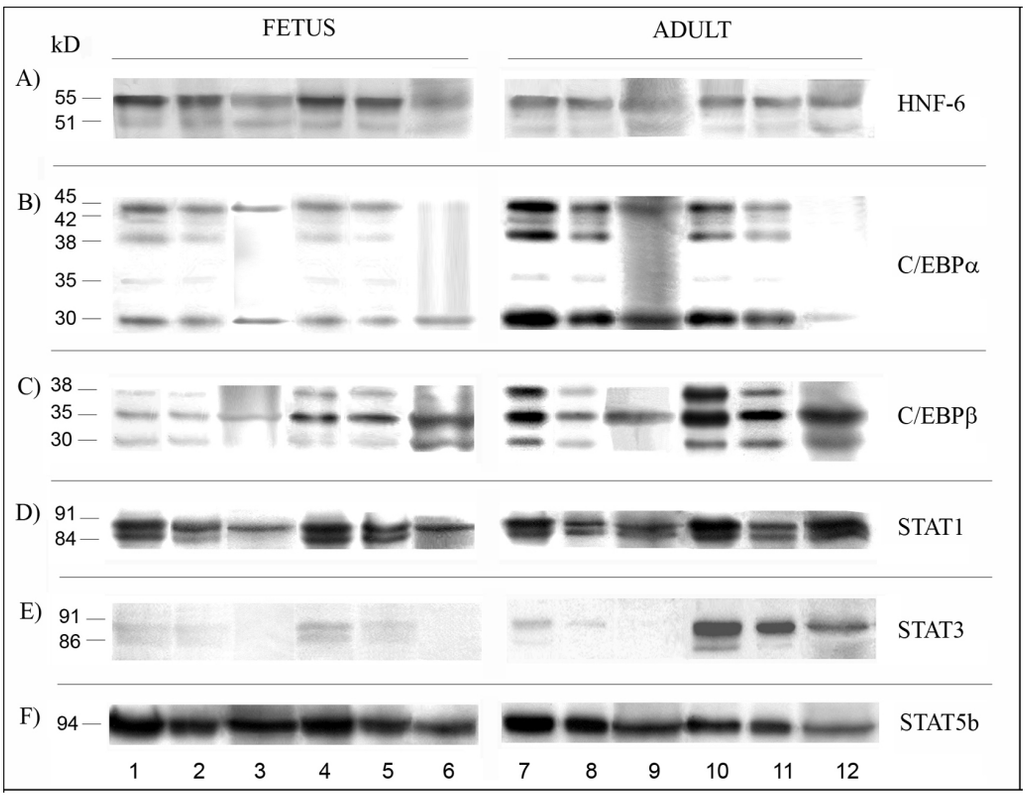


**Fig. 3.** Western blot analysis of nuclear matrices (NM), internal nuclear matrix proteins (INM), and INM purified by DNA-affinity chromatography from fetal and adult livers. Proteins (20 μg) were separated by SDS-PAGE in a 12% gel, electro-transferred onto PVDF membranes, and incubated with polyclonal rabbit antibody raised against rat HNF-6, C/EBPα, C/EBPβ, STAT1, STAT3, and STAT5b proteins. The antigen-antibody complexes were visualized by the ECL detection system. Equal quantities (5 mg) of INM were applied to the DNA affinity column, eluted with 1 M KCl, and probed with the indicated antibodies. Lanes 1 and 7 – control NM; 2 and 8 – control INM; 3 and 9 – control INM eluted with 1 M KCl; 4 and 10 – NM obtained 12 h after AP induction; 5 and 11 – INM obtained 12 h after AP induction; 6 and 12 – INM obtained 12 h after AP induction and eluted with 1 M KCl. Fetal and adult samples are indicated.

ing activity (Fig 3B), increased binding of the 35 kDa C/EBPβ isoform, appearance of a 30 kDa C/EBPβ isoform (Fig. 3C), and slightly decreased STAT5b binding activity (Fig. 3F). Increased binding of the 84 kD isoform of STAT1 and STAT3-associated promoter-binding activity was detected in the adult AP nuclear matrix. No DNA-binding activity of these proteins was observed in the fetus. These findings lend experimental support for involvement of the predicted proteins in the regulation of $\alpha_2$M gene activity. Thus, proteins hypothesized to participate in $\alpha_2$M gene transcription *in vivo* were apparently confined to the nuclear matrix.

Interaction of nuclear matrix proteins with MARs is important not only for chromatin organization, but also for gene regulation. According to the proposed model (Sjakste and Sjakste, 2001; Ostermeier et al., 2003), the nuclear matrix facilitates transcription by ensuring the proper spatial organization of transcriptionally active proteins and gene regulatory elements. Using MAR-Finder and WebSIDD algorithms, we performed a search for potential MAR elements up- and down stream from the $\alpha_2$M gene promoter (-852/+12). The 7763-bp-long (-4528/+3235) 5' region of the $\alpha_2$M gene, containing two introns and three exons, was analyzed. Computer analysis revealed the potential presence of one MAR in the 5' region upstream from

the coding sequence (at -3735/-3535 by MAR-Finder and -3651/-3607 by WebSIDD) and two putative MARs within the first intron (at +961/+1261 and +1311/1461 by MAR-finder and at +1180/+1181 and +1350/+1381 by WebSIDD). The potential MARs overlapped in two independent searches. Identification of potential MAR sequences in the 5' and first intron regions of the $\alpha_2$M gene, together with the experimentally determined dynamic associations of activated $\alpha_2$M gene promoter-binding transcription factors on the nuclear matrix, point to an *in vivo* role for the nuclear matrix as a transcriptional facilitator that responds to gene up-regulatory signals.

## DISCUSSION

Comprised of the spherical peripheral nuclear lamina and the internal nuclear matrix, the nuclear matrix represents an extensive network of essentially proteinaceous fibers spreading throughout nuclear space that serves as a backdrop for the functional assembly of nucleic acids and proteins (Davie, 1995; Nickerson et al., 1995; Tsutsui et al., 2005). At present, the nuclear matrix is considered to be an active participant in many nuclear functions (Berezney and Wei, 1998; Stein et al., 2003). With the detection of nuclear matrix-associated transcription factors (van Wijnen et al., 1993; Bidwell et al., 1993; Nickerson et al., 1995; Nardozza et al., 1996; Lindenmuth et al., 1997; Poznanović et al., 1999; Dinić et al., 2000), the initial suggestion that the nuclear matrix is involved in transcription was put forward (Getzenberg, 1994; Lindenmuth et al., 1997; Sjakste and Sjakste, 2001). Based on accumulated experimental evidence and more recent research in proteomics, a view has been put forward according to which the activity of a protein often depends on its cellular localization. Thus, it is held that many proteins are shuttled between different compartments as a form of regulation at the cellular level. Moreover, this view emphasizes that the distribution of a regulatory protein, rather than its absolute abundance, is an important aspect of its appropriate action. In keeping with this, the functioning of a protein cannot be considered in isolation, since it is frequently dependent on interactions with other proteins. Only after examining protein-protein interactions can definitive information about the function of a protein be obtained. For this study, our working hypothesis was that gene regulatory protein functions *in vivo* are connected and coordinated by interactions with protein components of the nuclear matrix.

We here demonstrate that transcription factors HNF-6, C/EBPα, C/EBPβ, STAT1, STAT3, and STAT5b, involved in regulation of the $\alpha_2$M gene (which responds to developmental and inflammation-associated signals), establish dynamic interactions with the rat liver nuclear matrix and show that different nuclear matrix-activated transcription factor association profiles are synchronous with different states of $\alpha_2$M gene expression. Moreover, we observed that HNF-6 and STAT5b are more abundant in the nuclear matrix of the fetus than in that of the adult, while the adult nuclear matrix is enriched in C/EBPα and C/EBPβ compared to the fetus. The $\alpha_2$M gene promoter binding activity for HNF-6, C/EBPα, and STAT5b was highest in the basal state in the fetus and the adult. Induction of the AP response was characterized by increased C/EBPβ and STAT1 promoter binding in the fetus and the adult, while STAT3 emerged as the putative regulator of increased $\alpha_2$M gene transcription during the AP response in the adult. We suggest that the observed dynamic changes in localization of activated transcription factors in the internal nuclear matrix compartment reflect the role the nuclear matrix assumes in concentrating and coordinating the spatial and temporal organization of gene regulatory proteins. Identification of presumed MAR elements in the 5' region of the $\alpha_2$M gene lends additional support for involvement of the nuclear matrix in the establishment of optimal conditions for efficient transcription. Since transcription factor-binding sites often reside between MAR elements (Boulikas, 1994) and because MARs possess enhancer activity (Sjakste, 1993), through multiple interactions – with DNA elements such as MAR sequences and with transcription factors – the role of the nuclear matrix can be expanded to a molecular platform that spatially and temporally coordinates all participants

in the transcription process (Lemon and Tjian, 2000; Nickerson, 2001; Sjakste and Sjakste, 2001; Fackelmayer, 2004).

A considerable challenge in investigations of the regulation of transcriptional networks is to obtain an understanding of the molecular basis of the orchestration of transcriptional events that are interdependent, but at the same time separated on different chromosomes. Protein-protein interactions between components of multiprotein complexes and transcription factors are increasingly coming into focus and illustrate the true complexity of gene transcription (Schrem et al., 2002). The nuclear matrix has an important place in resolving these questions, since chromatin organization and remodeling components, as well as transcription factors, have been found to be associated with the nuclear matrix.

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# ДИНАМИЧКЕ АСОЦИЈАЦИЈЕ ТРАНСКРИПЦИОНИХ ФАКТОРА СА НУКЛЕАРНИМ МАТРИКСОМ ЈЕТРЕ ПАЦОВА СУ ФУНКЦИОНАЛНО ПОВЕЗАНЕ СА ДИФЕРЕНЦИЈАЛНОМ ГЕНСКОМ ЕКСПРЕСИЈОМ АЛФА-2-МАКРОГЛОБУЛИНА

СВЕТЛАНА ДИНИЋ, МИРЈАНА МИХАИЛОВИЋ, СВЕТЛАНА ИВАНОВИЋ-МАТИЋ, АЛЕКСАНДРА УСКОКОВИЋ, НЕВЕНА ГРДОВИЋ, МЕЛИТА ВИДАКОВИЋ и Г. ПОЗНАНОВИЋ

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Циљ рада је испитивање учешћа једарног матрикса у регулацији транскрипције гена за алфа-2-макроглобулин током развића јетре пацова и акутно фазног одговора (АФО). Након ДНК афинитетне хроматографије протеина унутрашње мреже једарног матрикса феталне и адултне јетре, у базалним и АФО условима, са промоторским елементом гена за $\alpha_2$M (-852/+12) и имуноблот анализе, идентификоване су динамичке асоцијације транскрипционих фактора укључених у регулацију експресије гена за $\alpha_2$M са једарним матриксом. HNF-6, C/EBPα, STAT5б су укључени у регулацију базалне експресије гена за $\alpha_2$M, док C/EBPβ, STAT1, STAT3 посредују у регулацији експресије овог гена током АФО. Описане интеракције доприносе разумевању предложених механизама којима се транскрипциони фактори усмеравају ка циљним регулаторним елементима ДНК.