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BINDING OF RAT LIVER NUCLEOPROTEINS TO THE DISTAL REGULATORY ELEMENT OF THE α_1-ACID GLYCOPROTEIN GENE

G. POZNANOVIĆ, SVETLANA IVANOVIĆ-MATIĆ, VESNA GRUJIĆ, ILIJANA GRIGOROV, DESANKA BOGOJEVIĆ and M. PETROVIĆ

Department of Molecular Biology and Biochemistry, Institute for Biological Research, 11060 Belgrade, Yugoslavia

Abstract - The binding of insoluble nuclear matrix and soluble nucleoproteins to DRE, the distal responsive element (-5300/ -5150) that is required for the transcriptional regulation of the rat α_1-acid glycoprotein (AGP) gene, was examined in the basal state when AGP transcription was low, and during the acute-phase response when its transcription was increased. By South-Western analysis of nuclear matrix preparations it was established that as a result of the acute-phase response, in addition to binding to a 40 kDa protein (p40), the distal responsive element exhibited a considerably increased binding to p45, and a slightly less prominent but enhanced binding to p29, p35, p37 and p55 proteins in relation to the control. South-Western analysis of the soluble nucleoprotein preparation revealed an increased binding to p35, p37 and p45, and an unchanged binding to p29 and p70. The presence of polypeptides with apparently identical molecular masses in the different nuclear components points to the existence of dynamic interactions between nuclear compartments that are related to the functional properties of the nucleus.

Abbreviations - AGP, α_1-acid glucoprotein; DRE, distal responsive element.

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INTRODUCTION

After a systemic injury an increase of a subset of serum proteins, designated as the acute phase reactants, is raised during the ensuing acute-phase response (Kushner 1982). α_1-acid glycoprotein (AGP) is one of the major acute-phase proteins in the rat. Its elevated synthesis in the liver is primarily regulated at the transcriptional level (Alam and Papaconstantinou 1992), and is essentially mediated by interleukins-1 and -6, tumor necrosis factor - α and glucocorticoids (Baumann and Gauldie 1994). AGP gene expression depends on the interaction of CCAAT-enhancer binding proteins (C/EBP) to 5′ upstream regulatory *cis*-elements: the glucocorticoid response element (GRE) - 121/-62, and the distal cytokine responsive element (DRE)-5300/-5150 (Ray and Ray 1994).

It was previously shown (Ševaljević *et al.* 1995) that during the acute-phase response-related elevated transcription of the rat haptoglobin gene, p29, p35, p45, p68 and p70 from the soluble nucleoprotein preparation or nuclear extract, bound with an increased affinity to the haptoglobin *cis*-acting ABC element (-165/-56) that is crucial for all hormone regulatory functions (Marinković and Baumann 1990). Furthermore, p55 (pI 6.0) and the lamins mediated the increased binding of a 38 bp adenine tract lying 147 bp 5′ upstream from the haptoglobin *cis* - element to the nuclear matrix (Poznanović *et al.* 1994). The latter findings supported the postulated assertion that certain DNA-binding nuclear matrix proteins influence the association of mobile *trans*-acting factors with the *cis*-acting sequence by localizing or concentrating them (Dworetzky *et al.* 1992; Das *et al.* 1993; Luderus *et al.* 1994).

In this communication we identified a set of AGP gene DRE-binding proteins in the nuclear matrix, the insoluble, three-dimensional proteinaceous nuclear network, and in the transcriptionally active soluble nucleoprotein fraction or the nuclear extract (Gorski *et al.* 1986). These proteins bound the DNA when AGP gene transcription was low, and after its stimulation as a result of the acute-phase response that was triggered by a turpentine-induced inflammation. The two nucleoprotein preparations contained common DRE-binding

proteins, as well as proteins that were specific for each fraction, suggesting that these distinct nuclear components established different and dynamic associations before and during the acute-phase response.

MATERIALS AND METHODS

Animals and treatment

Turpentine is a commonly acceptable agent for induction of the acute-phase reaction (Baumann *et al.* 1984). The response was elicited by two simultaneous s.c. injections of turpentine oil (*µl/g* body weight) dorsally into the paws of three month old male albino rats. The animals were killed 24 *h* after the initial treatment. The control animals were administered saline.

Nuclear matrix preparation

The nuclear matrix was isolated from purified rat liver nuclei isolated as described previously (Kaufmann and Shaper 1984). The nuclear matrix was isolated from purified rat liver nuclei essentially by the high-salt extraction procedure (Nakayasu and Berezney 1991). All of the buffers contained 1 mM phenylmethylsulfonyl fluoride.

Nuclear extract preparation

Rat liver nuclear extracts containing positive transcription factors were isolated as described (Gorski *et al.* 1986).

Nuclear transcription assay

In vitro transcription, hybridization and washing of filters were performed according to Vannice *et al.* (1984). The *in vitro* [α^{32}]UTP-labeled RNA (2×10^8 d.p.m.) isolated from 10^8 nuclei of control and acute-phase rats, was hybridized to nitrocellulose filters containing 3 µg of linearized denatured plasmid DNA containing inserts complementary to rat mRNA for AGP and pBR322. The transcription assays were quantified by scanning the autoradiograms in a linear range. The transcriptional activity of the AGP gene was calculated as a percentage increase of the value obtained for control rats (100%). The values were corrected for background binding to pBR322.

South-Western analysis

After sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Sambrook *et al.* 1989), proteins were either stained with Coomassie brilliant blue R-250 or transferred to nitrocellulose filters by electroblotting. South-Western analysis was performed according to Bowen *et al.* (1980) by probing with 10^6 cpm/lane of [α^{32}P]dCTP-labeled restriction fragment DRE. The washed filters were exposed to X-film.

DNA procedures

DNA preparation and radioactive labeling by random priming were performed according to Sambrook *et al.* (1989). The plasmids encoding AGP cDNA in pBR322 and DRE in pUC13 were obtained from Dr. Heinz Baumann from the Dept. Mol. Cell. Biol, Roswell Park. Inst., Buffalo, N.Y., U.S.A.

RESULTS AND DISCUSSION

Coomassie-staining of the nuclear matrix (Fig.1, lane 1) and the nuclear extract (Fig.2, lane 1) revealed their unique and complex protein composition. Although the acute-phase response led to a 2.1-fold increase in the relative transcription rate of the AGP gene, as established by an *in vitro* nuclear run-off assay, the respective protein profiles remained virtually unchanged, without any observable qualitative difference (Figs 1 and 2, lanes 2). In contrast to the heterogenous protein composition of these nuclear fractions, South--Western analysis of the nuclear matrix and nuclear extract revealed that a limited set of proteins bound the distal AGP gene *cis*-element. Prominent DNA binding was observed to p40, and to a lesser extent to p45 in the nuclear matrix isolated from the controls (Fig. 1, lane 3). The acute-phase response was accompanied, in addition to the binding of DRE to p40, by a significantly increased binding to p45, and a new but slighter binding to p29, p35, p37 and p55 (Fig. 1, lane 4). In control preparations of the nuclear extract, besides an unchanged binding to p29, p50, p68, p70, p97 and p116 (Fig. 2, lane 3), South-Western analysis revealed an increased binding of DRE to p35, p37 and p45 during the acute-phase response (Fig. 2, lane 4).

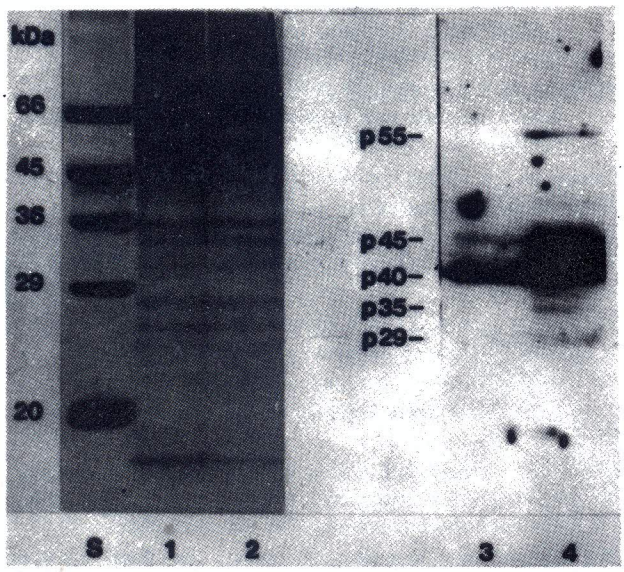

Fig. 1. Coomassie-profiles (lanes 1 and 2) and autoradiograms after South-Western analysis (lanes 3 and 4) of nuclear matrix proteins isolated from control rats (lanes 1 and 3) and 24 *h* after the onset of the acute-phase response (lanes 2 and 4). The molecular weight standards (lane S) were bovine albumin (66 kDa), egg albumin (45 kDa), glyceraldehyde 3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), and trypsin inhibitor (20 kDa).

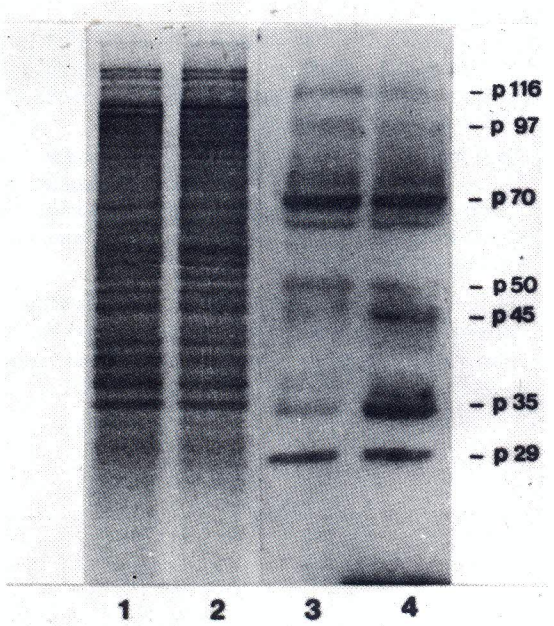

Fig. 2. Coomassie-profiles (lanes 1 and 2) and autoradiograms after South-Western (lanes 3 and 4) analysis of nucleoprotein extracts isolated before (lanes 1 and 3) and 24 *h* after the onset of the acute-phase response (lanes 2 and 4).

On the basis of the DRE-binding affinity as a function of the acute-phase response, the DRE-binding proteins in these nuclear preparations were divided into two groups. To the first group belong inducible proteins that displayed an increased binding for DRE as a result of the acute-phase response. These proteins are assumed to play a role in the acute-phase response-promoted increase of AGP gene transcription and include p29, p35, p37, p45 and p55 from the nuclear matrix, and p35, p37 and p45 from the nuclear extract. The second group encompasses proteins that bound DRE constitutively, with an unchanged affinity, regardless of the transcriptional activity of the AGP gene. Within this group are p29, p50 and the p68-p116 proteins exclusively from the nuclear extract, indicating that the majority of DRE-binding proteins that partitioned in the nuclear matrix were inducible.

The existence of specific DRE-binding protein profiles that characterize each nuclear fraction essentially rules out the possibility of significant contaminations of nuclear matrix and soluble nucleoprotein preparations with their respective protein components. Therefore, the finding of shared DRE-binding proteins in distinct nuclear components could reflect different and dynamic associations of these components as a result of posttranslational protein modifications during the acute phase response or, operationally, altered protein solubilities. In agreement with this is the finding that p70 in the soluble nucleoprotein fraction shares charge, size and epitopes with the nuclear matrix constituent lamin A (Ševaljić and Šekularac 1993). Likewise, the existence of DNA-binding activities related to different *trans*-factor families that reside in the nuclear matrix (Wijnen *et al.* 1993) was illustrated by the observed nuclear matrix-associated p35 DRE binding activity. This could have resulted from the association of p35, a presumed member of the C/EBPβ transcription factor family (Won and Baumannn 1991), with nuclear matrix proteins during the acute-phase response. Finally, a structural and functional homology between p29 and HMG1 was recently established (unpublished results). The findings presented here support the assertion that the regulation of gene expression is accomplished through an interplay between the nuclear matrix and the specific three-dimensional organization of the genome (Getzenberg, 1994).

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