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DISTRIBUTION OF THE HAPTOGLOBIN GENE 05′ FLANKING DNA-BINDING PROTEINS IN THE NUCLEAR MATRIX.

Vesna Grujić, Svetlana Ivanović-Matić and G. Poznanović, *Department for Molecular Biology and Biochemistry, Institute for Biological Research,* 11060 Belgrade, Yugoslavia.

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The nuclear matrix, a spherical proteinaceous structure consisting of the peripheral nuclear lamina with nuclear pore complexes, an extensive internal filamentous network and residual nucleoli, is obtained after the extraction of chromatin, soluble proteins and the lipoprotein nuclear membrane. DNA loop attachment to the matrix is accomplished through contacts of specific DNA sequences, the matrix association regions (MARs) that delineate different genes and play a role in gene expression (Laemmli *et al.* 1992; Getzenberg 1994). In addition to MARs, there are other unique sequences on the DNA that likwise bind to the nuclear matrix (Hakes and Berezney 1991). This binding increases during elevated gene transcription (Brotherton *et al.* 1991; Sun *et al.* 1992; Poznanović *et al.* 1992; 1994; 1996) as a result of a remodeling of the thee-dimensional conformati on of the points of contact on the matrix due to protein redistribution and posttranslational modifications (Poznanović *et al.* 1995; 1996).

In this work the binding of the haptoglobin gene restriction fragment -541/-146 to the peripheral lamina and the internal matrix, the structural components of the nuclear matrix, was examined by South-Western analysis. Compared to other sequences within the haptoglobin gene 5′ flanking region, the nonhistone protein component of the nuclear matrix demonstrates the highest binding potential for this particular region during the acute phase response (APR)-promoted elevation of haptoglobin gene transcription (Poznanović *et al.* 1992, 1994a). Our aim was to obtain preliminary information about the changes in distribution of the DNA-binding proteins in the matrix as haptoglobin gene transcription progresses from the basal to an elevated state as a result of the APR.

The APR was induced by a *s.c.* injection of turpentine, and the rats were killed 24 *h* after the treatment. Hepatocyte nuclei were isolated as described (Kaufmann and Shaper 1984) in the continual presence of 1 mM Na-tetrathionate. Nuclear matrices were isolated by nuclease digestion, followed by extractions with 0.2 M $(NH_4)_2SO_4$ (AmS) and Triton X-100 buffered solutions (Belgrader *et al.* 1991). The internal matrix was isolated by reduction of the nuclear matrices with 40 mM dithiothreitol in AmS buffer (Luderus *et al.* 1992). The peripheral laminas were prepared from the nuclei in which disulfide bond formation was prevented by the inclusion of 10 mM iodoacetate in all of the buffers (Stuurman *et al.* 1990); the isolated nuclei were subsequently extracted with 10 mM β-mercaptoethanol in 1.6 M NaCl buffered solution. After sodium dodecyl sulfate polyacrylamide gel electrophoresis (Sambrook *et al.* 1989), total nuclear matrix, peripheral lamina and internal matrix proteins were electroblotted onto nitrocellulose filters. South-Western analysis (Bowen *et al.* 1980) was performed by probing the filters with 5×10^5 cpm/lane of the P^{32}-labeled haptoglobin fragment -541/-146, followed by autoradiogrphy overnight. DNA procedures were performed as described (Sambrook *et al.* 1989).

The nuclear matrix and its structural components, the peripheral lamina and the internal nuclear matrix framework, were isolated and haptoglobin gene restriction fragment -541/-146 binding to their protein constituents (Fig. 1A) before and 24 *h* after the infliction of the APR assessed by South-Western analysis. The lamins (62-68 kD), 55, 53, 40, 35, 33 and 29 kD polypeptides in the control total nuclear matrix bound the DNA (Fig. 1B, lane 1). In the APR nuclear matrix (Fig. 1B, lane 2), except for the 33 kD polypeptide, the same proteins bound more DNA. The peripheral lamina that retained about 2/3 of the total nuclear matrix protein displayed a notable increase of DNA binding (Fig. 1B, lane 3), presumably due to its enrichment with DNA-binding proteins. As a result of the APR the DNA-binding affinity of the lamina proteins was further enhanced (Fig. 1B, lane 4). However, the overall DNA-binding pattern was very similar to the total nuclear matrix protein DNA-binding profile with the following exception: the 57 and 45kD DNA-binding proteins found in the peripheral lamina were not evident in the total nuclear matrix preparation, while the prominant 40 kD DNA-binding protein in the total nuclear matrix was absent from the lamina preparation. In the APR peripheral lamina (Fig. 1B, lane 4), the greatest increase of DNA-binding affinity was exhibited by proteins in the 30-35 kD region and to a much lesser extent by several proteins in the 45-57kD region. The most prominant DNA-binding protein in the control internal matrix (Fig. 1B, lane 5) was a 45 kD protein, and to a lesser extent polype-

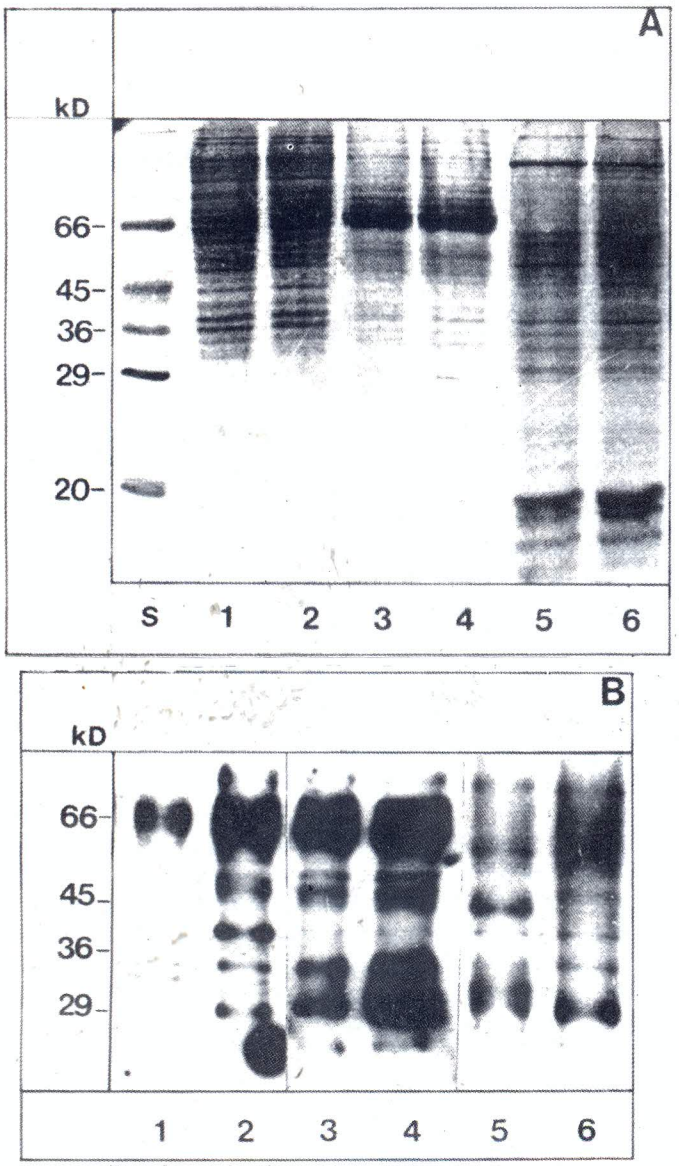

Fig. 1. Control (lanes 1, 3 and 5) and APR (2, 4 and 6) total nuclear matrix (1 and 2), peripheral lamina (3 and 4), and internal matrix proteins (5 and 6). A - Coommassie-profiles, B - autoradiograms after DNA binding.

ptides in the 30-33 kD region and a 62 kD protein. The APR led to disappearance of DNA-binding to the 45 and 33 kD proteins, and to appearance of 62-68, 55, 40, 35 and 30 kD DNA-binding proteins (Fig. 1B, lane 6). These results revealed a qualitative, rather than quantitative difference between the control and APR internal matrix DNA-binding protein patterns. This feature was apparently obscured in the total nuclear matrix where the internal matrix framework proteins contributed to only about 1/3 of the total protein.

The presented results show that the DNA-binding potential of the underlying nuclear proteinaceous foundation undergoes a change that accommodates it to the requirement of increased haptoglobin gene transcription during the APR. This is illustrated by the observed increased binding affinity of nuclear matrix proteins for the haptoglobin gene restriction fragment -541/-146. Futhermore, increased transcription appears to be associated with an exchange of proteins between different nuclear matrix structures of soluble compartments of the nucleus, most notably of the prominent control internal matrix 45 kD DNA-binding protein. Further experiments using antibodies to specific DNA-binding proteins are required in order to provide a clearer picture of the implied molecular reorganization of the nuclear matrix at the points of contact with DNA during elevated haptoglobin gene transcription.

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