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THE 70 kD RAT LIVER NUCLEOPLASMIC PROTEIN BINDING HORMONE RESPONSIVE ELEMENT OF RAT HAPTOGLOBIN GENE SHARES SIZE, CHARGE AND EPITOPES WITH LAMIN A

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Summary:The 70 kD rat liver nucleoplasmic protein was shown previously to display binding affinity for hormone responsive element of rat haptoglobin gene which markedly increased during the acute-phase reaction. In this work the possibility of its structural homology to lamin-type intermediate filament proteins is assessed. The results of two-dimensional Southwestern and Western analyses pinpointed to size, charge and epitope homology between the 70 kD rat liver nucleoplasmic protein binding the hormone responsive element and the lamin A constituent of nuclear matrix structure.

A transient increase in transcription of liver-specific genes coding for a subset of plasma proteins, the acute-phase (AP) reactants, in response to acute tissue injury has been widely explored in studies on the molecular mechanisms regulating gene expression. Haptoglobin (Hp) is a prominent AP protein displaying pleotropic activities, the main of which are related to binding and clearance of hemoglobin (1-3). A severalfold enhancement of the plasma Hp concentration during the AP reaction is achieved through hormonal stimuli-mediated increase in the rates of Hp gene transcription and synthesis of the corresponding mRNA and protein (4,5). The mediators promoting full expression of the Hp gene in rat liver cells have been identified as interleukin-6 (IL-6), IL-1 and glucocorticoids (6,7). It has been shown by transfection experiments that the sequence conferring IL-1, IL-6 and dexamethasone responsiveness to the reporter gene lies in the promoter proximal region at position -165 to -55 (8,9). This regulatory sequence, termed ABC element, consists of three separate, cooperatively interacting subsequences which integrated into an expression vector do not function identically in rat and human hepatoma

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cells most likely due to differences in composition of trans-acting factors (9). This, together with available data addressing identification of transcription factors, argued for the possibility of a distinguishable composition of proteins regulating the rat Hp gene transcription *in vivo* and in different hepatoma cell systems. Recent studies pinpointed to an AP response-related increase in the affinity of several rat liver nucleoplasmic proteins to bind the hormone responsive element of the rat Hp gene (to be published). This group of proteins comprised a few members of C/EBP family and unidentified protein migrating with apparent Mr of 70 kD. The present work is focused on its characterization and identification.

MATERIALS AND METHODS

Adult male Wistar rats weighing 200-300g were used. The AP reaction was induced by s.c. injection of turpentine (μl *per* g b.w.) in the lumbar region.
The nuclear matrices were prepared from liver nuclei isolated in the presence of magnesium ions (Mg-nuclei) essentially as described by Kaufmann *et al.*, 1981 (10).
Rat liver nuclear extracts were prepared 0 and 12h after injection of turpentine as suggested by Gorski *et al.* (11).
Polyspecific antibodies to nuclear matrix proteins were obtained following the procedure of Ševaljević *et al.*, 1981 (12).
Western analysis was performed according to Towbin *et al.* (13).
Protein concentrations were determined after Lowry *et al.* (14).
The proteins were separated by one-dimensional (15) or two-dimensional (16) SDS-PAG electrophoreses and transferred onto nitrocellulose sheets in Hoefer TE 70 SemiPhor.
Southwestern analysis was performed after Bowen *et al.* (17).
Fragment of the Hp gene spanning from -165 to -56, kindly donated by Dr Heinz Baumann (Roswell Park Memorial Institute, Buffalo, NY), was used as the DNA probe. The fragment inserted into the Hinc II site of pUC 13 was isolated according to the standard procedure described by Sambrook *et al.* (15). DNA probe was (α-^{32}P)dCTP-labelled by the random priming technique (18).

RESULTS

Nucleoplasmic proteins from livers of control and turpentine-treated rats were separated by charge in the first and by size in the second dimension, blotted and probed with the labelled hormone RE of the rat Hp gene. Figure 1 reveals the presence of a spot at the position of 70 kD, pI 6.8 that was barely detectable for control and intense for the AP liver extracts. This, supported by the results of Coomassie blue staining which

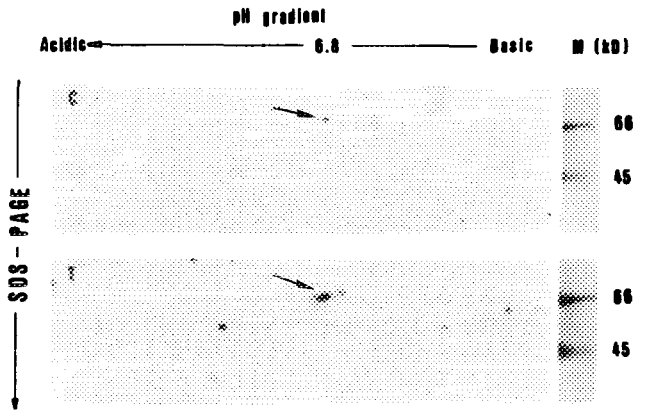

Figure 1. Two-dimensional Southwestern analysis of nucleoplasmic proteins.
Nucleoplasmic proteins from the control (C) and turpentine-treated (T) rat livers were separated in two-dimensional SDS-PAGE system, blotted and probed with (α-^{32}P)dCTP-labelled hormone RE of the rat Hp gene. The radioactive spot appearing at the position of 70 kD, pI 6.8 is indicated by an arrow.

revealed no difference between the electrophoretic profiles of the proteins from the two liver extracts (not shown), implicated the AP-related increase in affinity of the 70 kD, pI 6.8 protein for the probed DNA sequence. Electrophoretic characteristics of the protein forming the complex with the hormone RE corresponded to those of pI 6.8 nuclear matrix lamin A isoform (19,20). While the origin and properties of the 70 kD protein have not been investigated so far, the available data suggest that most, if not all, of the hormone RE-binding proteins migrating with Mr ranging from 20 to 40 kD could represent the members of C/EBP family of intermediate filament proteins (IFP). Taking into account that the lamins are the proteins of IF type as well (21-23), it was tempting to examine the possibility of structural homology of the hormone RE-binding 70 kD nucleoplasmic protein which by size and by charge resembled the lamin A to the corresponding lamina protein. To this aim nuclear matrix and nucleoplasmic proteins separated in two-dimensional SDS-PAGE system and transferred onto nitrocellulose sheets were probed with polyspecific anti-nuclear matrix protein antibodies. Silver staining of nuclear matrix proteins revealed the characteristic lamin pattern (19,20) where the spots in the 70 kD range at pI 6.8 to 7.2 and 5.7 corresponded to isovariant forms of lamins A and B, respectively, whereas lamin C was located below lamin A at

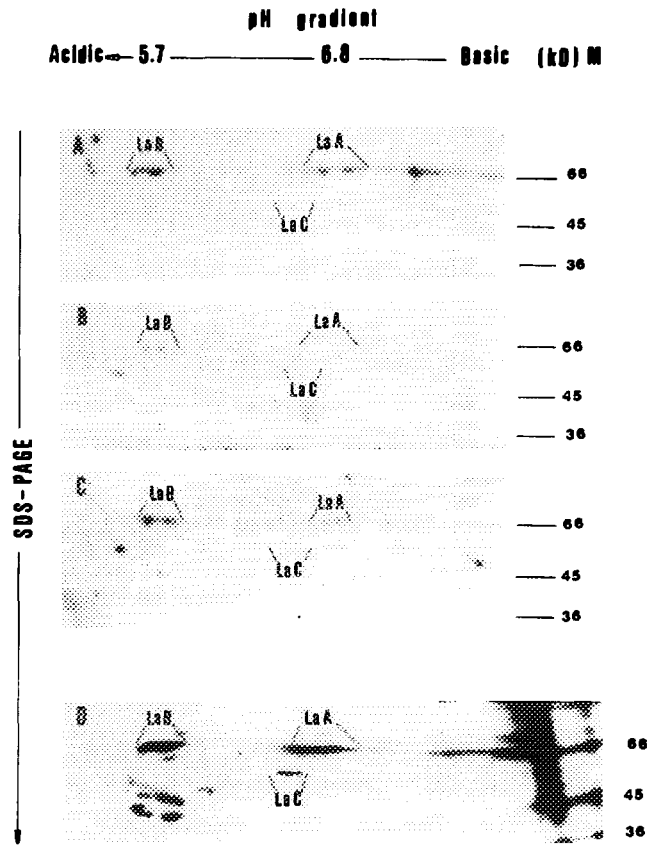

Figure 2. Two-dimensional Western analysis of nuclear matrix and nucleoplasmic proteins and silver-stained pattern of nuclear matrix proteins.
A-C. Nuclear matrix (A) and nucleoplasmic proteins from control (B) and turpentine-treated rat livers (C) were separated in two-dimensional SDS-PAGE system, blotted and probed with polyspecific anti-nuclear matrix antibodies. Bound antibody was visualised following treatment of the filters with ^{125}I-labelled secondary antibody; D. Nuclear matrix proteins from control liver were separated in two-dimensional SDS-PAGE system and visualised by silver staining. Location of the lamins A, B and C, as well as that of their nucleoplasmic homologs, is indicated by abbreviations: La A, La B and La C, respectively.

position of 63 kD (Fig. 2D). Figure 2A shows that lamins A and B were recognized by anti-nuclear matrix antibodies as well as that reaction with lamin C was below the limit of detection. Immunoblotting of the nucleoplasmic proteins from control liver with anti-nuclear matrix antibodies resulted in the formation of antigen-antibody complexes at the position of lamin B (Fig. 2B). Induction of the AP reaction was accompanied by intensification of the spot occupying the 70 kD, pI 5.7 region of the blot and appearance of new one at the position of 70 kD,

pI 6.8 which corresponded to that of the lamin A (Fig. 2C). This finding revealed structural homology between the pI 6.8 and 5.7 constituents of the 70 kD nucleoplasmic proteins binding the probed antibodies in an acute phase-dependent manner to the lamins A and B, respectively. Presence of the spot in the 70 kD, pI 6.8 region of the Southwestern blot (Fig. 1) being of much higher intensity for the AP than the control liver extracts suggested that the lamin A-like protein, unlike the nucleoplasmic homolog of the lamin B, displayed affinity of binding the hormone RE of the rat Hp gene which increased during the acute-phase reaction.

DISCUSSION

The present work pinpoints to the existence of the 70 kD nucleoplasmic proteins sharing size, charge and antigenic determinants with nuclear matrix lamins A and B. Unlike the nucleoplasmic homolog of the lamin B, that of the lamin A is capable of forming the complex with the hormone RE of the rat Hp gene. The complex between the same DNA probe and the lamin A itself, however, was not observed (to be published). The amount of the lamin A-like protein-hormon RE complex is remarkably higher for the AP than the control liver extracts which implies an acute phase-related higher-order structure modification of this protein. By analogy to transient disassembly of the nuclear lamina into soluble dimeric and tetrameric forms during mitosis (24), the 70 kD protein binding the hormone RE could represent soluble, highly modified form of the lamin A whose function has diverged from that of the parent molecule.

The lamins, the same as C/EBP (25), Jun/Fos (26) and ATF/CREB (27) basic-leucine zipper regulatory proteins, have been identified as the members of superfamily of intermediate-type fillament proteins (IFP), all of which have in common the conserved α-helical rod domain (21,28). Trans-acting factors identified so far, which regulate the Hp gene transcription through cis-acting sequences of its hormone RE, belong to the b-zip class of IFPs as well (29,30). Such a prominent involvement of IF proteins in regulation of transcription process argues for the possibility that the hormone RE-binding 70 kD nucleoplasmic protein, which by size, charge and antigenic determinants resembles the lamin A, could belong to the IFP group of transcription factors.

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