SPECIES-SPECIFIC INTERACTIONS BETWEEN NUCLEAR PHOSPHOPROTEINS AND DNA

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SUMMARY

A class of nuclear acidic proteins, associated with DNA and present in the chromatin of different cell types, has been isolated and resolved into separate components by electrophoresis in polyacrylamide gels. The electrophoretic patterns indicate that the fraction is highly heterogeneous and that the distribution of chromosomal proteins is tissue-specific. Many proteins in rat liver and kidney chromatin are phosphoproteins which rapidly incorporate ^{32}P-orthophosphate into phosphoserine and phosphothreonine residues. DNA-protein binding studies show that many nuclear phosphoproteins bind selectively to the DNA of the species of origin (rat). Binding to the DNA of a closely-related mammal (mouse) is also observed, but to a lesser extent. No binding is observed when rat phosphoproteins are mixed with the DNA's of widely-divergent species, such as calf thymus or pneumococcal DNA's.

This communication is concerned with the isolation, properties and function of a class of acidic proteins associated with the DNA in the interphase chromosomes of higher organisms. Many of these are phosphoproteins. Particular emphasis is placed on new evidence that many of the acidic, non-histone proteins of the cell nucleus can recognize and combine selectively with the DNA of the species from which they were prepared.

The preparation of chromosomal acidic proteins is based upon a new method of extraction (1, 2) which has been applied to nuclei and chromatin fractions (3) isolated from various tissues of the rat. Highly-purified nuclei were prepared by centrifugation through sucrose density barriers (2), and a differential extraction of nuclear proteins was then carried out.

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Saline-soluble components of the nuclei were removed by washing in 0.14 M NaCl. Histones and other acid-soluble proteins were extracted in 0.25 N HCl, leaving a residue which was treated with chloroform-methanol-HCl to remove lipids and phospholipids (2). The residue was resuspended in 0.1 M Tris (HCl) buffer, pH 8.4, containing 0.01 M EDTA and 0.14 M 2-mercaptoethanol, and the suspension was mixed gently with an equal volume of cold buffer-saturated phenol. The phosphoproteins move into the phenol phase, leaving the nucleic acids in the aqueous phase which was discarded. The phenol phase was dialyzed against 0.1 M acetic acid - 0.14 M 2-mercaptoethanol, and against a series of urea-containing buffers which restore the phenol-soluble proteins to the aqueous phase for further characterization (2). In the final stage of the preparation, the acidic proteins were dissolved in 0.1 M Tris (HCl), pH 8.4, containing 0.01 M EDTA and 0.14 M 2-mercaptoethanol. Aliquots of the solution were analyzed for total protein content (4), for alkali-labile phosphate (5), for phosphoserine and phosphothreonine (6), and for average amino acid composition (7). Phosphoproteins labeled with ^{32}P were isolated from liver and kidney nuclei of animals which had been injected 90 minutes earlier with ^{32}P-orthophosphate (2 mc/100 g rat body weight).

The separation and characterization of individual proteins in the extract was achieved by polyacrylamide gel electrophoresis. The procedure employed is based on the separation of protein-sodium dodecylsulfate complexes (8, 9), under conditions in which the distance of migration of individual proteins can be correlated with their molecular weights (9, 10). Details of the electrophoresis procedure, staining of the proteins bands with Amido black 10B, densitometry of the stained gels, and chemical and isotopic methods for determination of protein content and specific activity in individual bands are described elsewhere (2, 11). The electrophoretic banding patterns (Figure 1) indicate the presence of

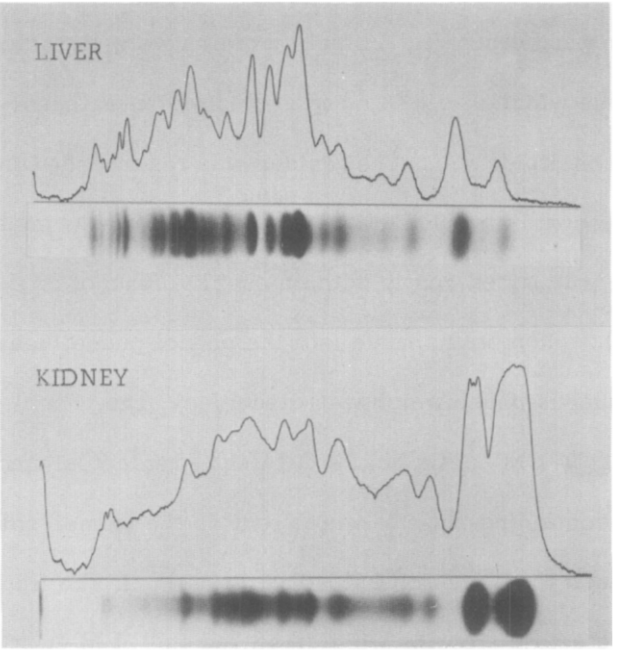

Figure 1. Electrophoretic separation of acidic proteins from rat liver and kidney nuclei. 220 μg of the phenol-soluble protein fraction, dissolved in 0.2 ml of 0.1 M Na phosphate, pH 7.4, - 0/1 % SDS, were applied to 10 % polyacrylamide gels and electrophoresed for 7 hours at 4.5 v/cm. The proteins were stained with Amido black 10B. A densitometer tracing is aligned over the corresponding banding pattern.

many proteins with a broad range of molecular weights. Densitometer tracings of the gel photographs are aligned with the banding patterns in the upper part of each figure. The distribution of the bands and their relative intensities are highly reproducible in different preparations from a given tissue, such as liver, but they vary from one tissue to another. A comparison of liver and kidney acidic proteins shows striking differences (Figure 1). Different electrophoretic patterns are obtained from brain and spleen nuclear phosphoproteins (2). Thus, tissue-specificity, which should be one characteristic of proteins concerned with the control of transcription in differentiated cells, is observed in the phenol-soluble protein fraction.

The presence of phosphate in the acidic nuclear protein fraction has been demonstrated by analysis of the phenol-soluble proteins for alkali-labile P content (5, 2). A limited acid hydrolysis of the proteins, followed by chromatography of the hydrolysate on Dowex-50 (6) reveals the presence of phosphoserine and phosphothreonine (2). The incorporation and release of phosphate by nuclear phosphoproteins in vivo is a major aspect of their metabolism (12 - 16). Studies of ^{32}P-orthophosphate incorporation into individual, electrophoretically separated proteins of rat liver and kidney nuclei have revealed clear differences in the extent of phosphorylation of different proteins from the same tissue (2). The pattern of phosphorylation of kidney and liver proteins also appears to be tissue-specific and hormone-dependent (11).

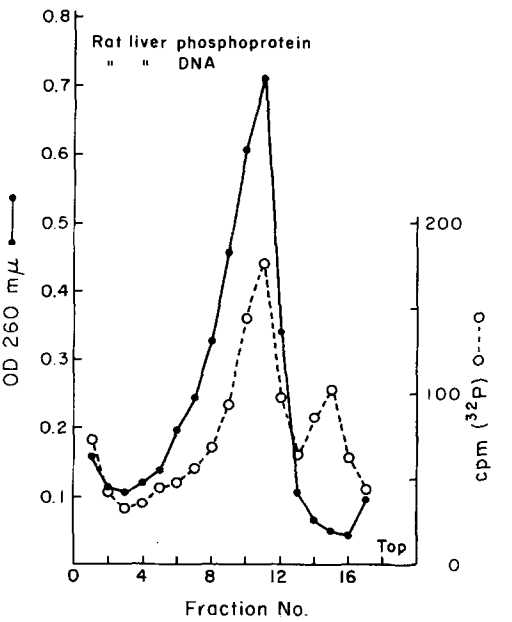

Figure 2. Separation of nuclear phosphoprotein - DNA complexes after reconstitution in vitro. A rat liver nuclear phosphoprotein fraction was "annealed" with rat DNA (2), layered over a 5 - 25% sucrose gradient, and centrifuged at 358, 000 x g for 2-1/2 hours. The position of DNA in the gradient is indicated by its absorption at 260 mμ (solid line). The distribution of isotopically-labeled phosphoprotein is shown by the dashed line.

^{32}P-labeled nuclear proteins have been used to study the specificity of interactions between phosphoproteins and DNA from different sources. The procedure is based on a slow "annealing" reaction in which samples of DNA and ^{32}P-labeled protein, each dissolved in 2 M NaCl - 5 M urea - 0.01 M Tris (HCl), pH 8.0, are mixed and dialyzed together against a progression of salt solutions of decreasing concentration (2). After removal of the urea by dialysis against 0.01 M Tris (HCl), pH 8.0 - 0.01 M NaCl, the samples were layered over a buffered 5 - 25 % sucrose gradient and centrifuged. The distribution of DNA in the gradient was determined by its absorption at 260 mμ, while the position of the phosphoproteins was indicated by ^{32}P-radioactivity. Binding of proteins to DNA is accompanied by a shift of ^{32}P-activity into denser regions of the gradient. Phosphoproteins not bound to DNA remain in the light, upper portion of the gradient.

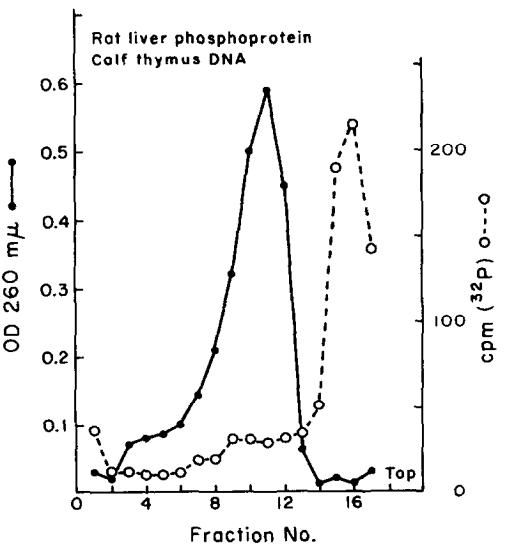

Figure 3. Evidence for selectivity in DNA binding by rat liver nuclear phosphoproteins. The phosphoprotein fraction, labeled in vivo by ^{32}P-orthophosphate, was mixed with calf thymus DNA and centrifuged in a 5-25 % sucrose gradient. Note that the isotopically-labeled protein (dashed line) fails to combine with DNA (solid line) and remains at the top of the gradient.

The application of this procedure to the separation of complexes of rat liver nuclear phosphoproteins and rat liver DNA is shown in Figure 2. A similar binding reaction was observed when rat kidney phosphoproteins were added to rat liver DNA (2). This type of association is relatively specific for the DNA of the species from which the phosphoprotein fraction was prepared. No comparable binding of rat liver phosphoproteins to calf thymus DNA (Figure 3) or to pneumococcal DNA (Figure 4) was observed. Similar specificities were noted for rat kidney phosphoproteins (2). While interactions between phosphoproteins and DNA's from widely-divergent species are minimal, binding of rat liver phosphoproteins to mouse liver DNA does occur. However, the extent of the binding reaction between rat proteins and mouse DNA is less than that observed when both the protein and the DNA come from the rat. For example, the protein to DNA ratio in the peak region of the gradient was 0.11 / 1 for rat DNA, and 0.08 / 1 for mouse DNA.

The proteins capable of DNA-association have been re-isolated from their DNA complexes by extraction in phenol, and then analyzed by polyacrylamide gel

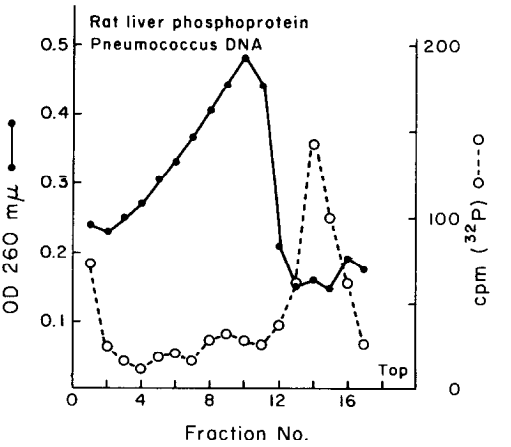

Figure 4. Evidence for selectivity in DNA binding by rat liver nuclear phosphoproteins. The ^{32}P-labeled phosphoprotein fraction was mixed with pneumococcal DNA and centrifuged in a 5-25 % sucrose gradient. Note that the isotopically-labeled protein (dashed line) fails to combine with the bacterial DNA (solid line) and remains at the top of the gradient.

electrophoresis. Many, but not all, of the original protein bands were found to be present in the complex, the results depending on the source of the phosphoprotein (2).

Our results show that different nuclear types of the same organism contain varying populations of acidic proteins with specific DNA-affinities. This is in accord with the recent report (17) that a small fraction of the non-histone protein of rat liver chromatin binds selectively to columns of rat liver DNA-cellulose. The significance of this specificity of DNA-associated proteins to differential transcription of the genome in diverse cell types remains to be determined.

Acknowledgements

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