

Tissue-specific Chromosomal Non-histone Protein Interactions with DNA*

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A group of chromosomal non-histone proteins with affinity for DNA (NP) was isolated from rat liver and Novikoff hepatoma. This fraction, which represents less than 5% of the total chromatin protein content, binds preferentially to unique, double-stranded sequences of fractionated homologous DNA. The interactions are strong at low ionic strength ($K_m = 6.7 \times 10^{-7}$ M) and decrease with rising salt concentration. Complexes of the NP protein fraction with homologous DNA are immunologically tissue-specific. As determined by microcomplement fixation, the NP proteins in Novikoff hepatoma are associated with the transcriptionally active, diffuse fraction of chromatin.

Chromosomal non-histone proteins exhibit heterogeneity and tissue specificity which is expected of proteins involved in gene regulatory capacity. Evidence has accumulated in the literature linking changes of chromosomal protein turnover, phosphorylation, specificity, and other properties to the stimulation of genetic transcription by hormones, mitogens, carcinogens, etc. Chromosomal non-histone proteins which can bind to DNA were described by several investigators (1-8). Considerable differences in the DNA binding properties and heterogeneity of these proteins reported in the literature indicate that there may be a family of proteins in the nucleus which can bind to the DNA with a variable degree of specificity. Wakabayashi *et al.* (7, 9, 10) and Chiu *et al.* (8) reported a group of non-histone proteins capable of forming complexes with homologous DNA which were tissue specific by immunological criteria. In this paper, we described some additional properties of the DNA-binding chromosomal proteins of rat liver and Novikoff hepatoma.

MATERIALS AND METHODS

Isolation of DNA-binding Proteins.—The fractionation scheme developed in our laboratory (8) was followed. This scheme is based on the solubility properties of chromosomal proteins in 5 M urea at three different pH values and salt concentrations. The first extraction is performed at relatively low ionic strength at pH 7.6 and it removes the

bulk of proteins which are not firmly associated with DNA in chromatin. The second extraction at high ionic strength and relatively low pH (5.0) takes the advantage of the solubility of histones under these conditions. The DNA-binding non-histone proteins are poorly soluble at this pH, which is near their isoelectric point, and remain with the residual chromatin pellet. They can be solubilized by increasing the pH to 8.0 in the final extraction step.

Briefly, isolated rat liver or Novikoff hepatoma chromatin (11) was gently homogenized in 5.0 M urea containing 50 mM sodium phosphate buffer, pH 7.6. After stirring for 2 to 3 hours, the mixture was centrifuged at 20,000 $\times$ g for 30 min, and the pellets were extracted once more in the same manner. The combined supernatants contained the bulk of chromosomal non-histone proteins. Histones were removed from the remaining pellets by resuspending them gently in 5.0 M urea/2.5 M NaCl/50 mM sodium succinate buffer, pH 5.0 and centrifuging the viscous solution at 120,000 $\times$ g for 36 hours. DNA and associated non-histone proteins pelleted, while histones and small amounts of other proteins remained in the supernatant. Finally, the DNA-binding protein fraction was recovered by dissociation in 5.0 M urea/2.5 M NaCl/50 mM Tris-HCl buffer, pH 8.0 and centrifugation at 120,000 $\times$ g for 48 hours. The NP¹ protein-containing supernatants were pooled, concentrated by ultrafiltration (Amicon UM-2 membrane), and used in the described experiments.

DNA Protein-binding Studies.—DNA was isolated according to the modified procedure of Marmur (12, 13) and sodiated with carrier-free ³²P¹ using the method of Comstock (14). The chloramine-T technique described by Senoda and Schlamowitz (15) was used for the iodination of NP proteins.

The binding affinity of NP proteins to unfragmented DNA was studied by reconstituting ³²P¹-iodinated NP fraction to DNA and separating free labeled protein from the NP-DNA complex by sucrose

* The abbreviations used are: NP, DNA-binding chromosomal non-histone protein fraction; UP, chromosomal non-histone proteins soluble in urea at low ionic strength; HP, histones; SSC, 0.15 M NaCl/0.015 M sodium citrate; C₂A, concentration (moles/liter) $\times$ time (s).

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