TISSUE SPECIFICITY OF NON-HISTONE CHROMATIN PHOSPHOPROTEINS

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1. Introduction

The chromatin-associated proteins of eukaryotic cells are thought to play a role in the regulation of gene transcription. Although the basic chromatin proteins, or histones, have been studied the most extensively as potential gene regulators, it has been found that there are relatively few different types of histones which tend to be quite similar in different cell types and in different species [1]. Thus, histones do not seem to exhibit the heterogeneity or tissue specificity which would be expected of proteins involved in regulating the activity of specific genes [1, 2].

Among the non-histone chromatin proteins, the phosphoproteins have stimulated considerable interest as possible regulators of gene activity [3–6]. Although the phosphoproteins account for at least half of the non-histone chromatin protein in rat liver nuclei, they tend to aggregate into insoluble complexes, making subfractionation difficult. Consequently, previous studies have dealt with the properties of phosphoprotein fractions containing a mixture of protein molecules. The present experiments demonstrate a method for obtaining reproducible separations of these molecules employing acrylamide gel electrophoresis in the presence of the detergent sodium dodecyl sulfate (SDS). The results indicate that, consistent with their proposed role in gene regulation, the non-histone chromatin phosphoproteins are highly heterogeneous and exhibit characteristic patterns which are specific for different tissues.

2. Methods

Nuclei were isolated from 200 g of bovine thymus, liver, and brain by adapting the procedure of Lφvtrup-Rein and McEwen [7]. The non-histone chromatin phosphoprotein fraction was extracted and purified from these nuclei as described by Gershey and Kleinsmith [8]. Isolated phosphoproteins were labeled with γ-^{32}P ATP by incubating for 15 min in the presence of 25 mM $MgCl_2$ [5]. The reaction was stopped by adding solid urea to a final concentration of 4.0 M, followed by dialys at 4° against 0.01 M sodium phosphate (pH 7.0) containing 4.0 M urea and 0.1% β-mercaptoethanol. SDS was introduced by a subsequent dialysis at 20° against 0.01 M sodium phosphate (pH 7.0) containing 0.1% SDS and 0.1% β-mercaptoethanol. Electrophoresis was performed in a 10% SDS-acrylamide gel as described by Weber and Osborn [9]. Gels were sliced at 0.5 mm intervals and counted in Bray's scintillation fluid [10].

3. Results

Stained gels obtained by electrophoresis of chromatin phosphoprotein fractions from thymus, liver, and brain indicate that this protein fraction is quite heterogeneous (fig. 1). We have routinely been able to resolve a minimum of 26 bands in thymus, 27 bands in liver, and 23 bands in brain preparations. The overall patterns share many features in common, which might be expected since we are dealing with related proteins. However, each tissue has a unique, reproducible pattern, which differs quantitatively and qualitatively from the others. The differences are shown most strikingly when densitometer traces of the 3 gels are superimposed (fig. 2A). Due to the exceedingly complex nature of the electrophoretic patterns, we considered the possibility that some of the heterogeneity

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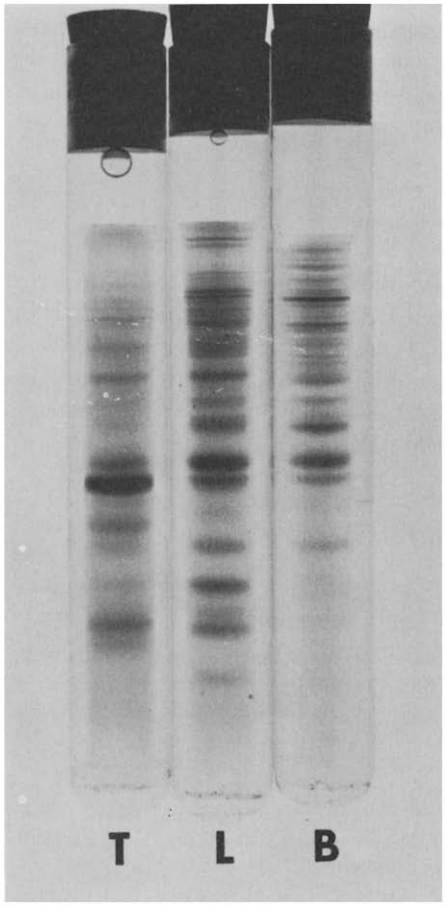

Fig. 1. SDS-acrylamide gel electrophoresis patterns of chromatin phosphoprotein preparations from bovine thymus (T), liver (L), and brain (B). Gels were stained with 0.25% Coomassie Brilliant Blue [9].

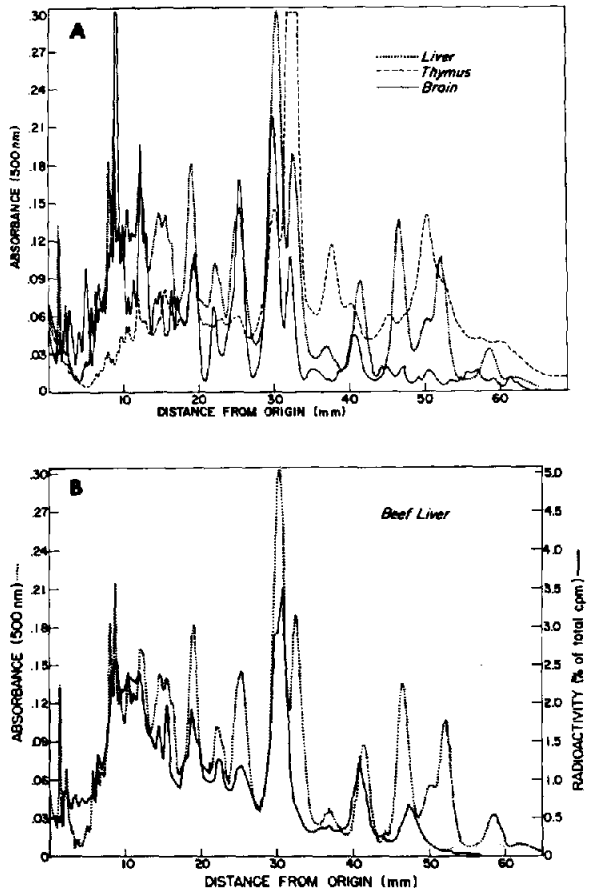

Fig. 2. (A) Densitometer tracings of the 3 gels pictures in fig. 1. The traces have been superimposed to point out the differences in pattern. (B) Distribution of radioactivity (——) in ^{32}P-labeled liver phosphoprotein compared with the distribution of protein bound stain (.....) in the same gel. Note that the majority of the protein peaks are radioactive.

represents degradation products. However, experiments in which protein preparations were allowed to sit for 24 hr at 4° or 22°prior to SDS treatment and electrophoresis yielded no significant alteration in the patterns.

The radioactivity profiles of phosphoprotein labeled with ^{32}P are quite complex. The specific activity of the bands varies considerably both within and between tissue types, and a detailed analysis will be reported elsewhere. However, it can be generally stated that nearly all of the bands contain ^{32}P, thus demonstrating that the observed heterogeneity does represent phosphorylated proteins (fig. 2B).

4. Discussion

The present experiments provide evidence for the heterogeneity and tissue specificity of the non-histone chromatin phosphoproteins. Although these findings are consistent with their proposed role in gene regulation, it might be argued that the observed number of components is still insufficient to account for the thousands of genes whose activity must be regulated. However, two points should be emphasized in this

regard. First, the SDS treatment dissociates any subunit structure which might be present in the native proteins, and thus during electrophoresis we are observing only denatured polypeptide chains. If subunit structure does exist, then the association of subunits in varying combinations would allow for additional diversity. Secondly, SDS-electrophoresis separates polypeptide chains only on the basis of size. If two polypeptide chains existed with the same amino acid backbone but with phosphate groups attached at different sites, they would still band together in this system. Thus each band could contain a family of polypeptide chains phosphorylated in different ways. Attempts to do autoradiography of peptide digests of these phosphoproteins have indicated that an extremely broad spectrum of radioactive peptides in fact does exist [11].

Considerable evidence has accumulated recently which suggests that the non-histone chromatin phosphoproteins are involved in the regulation of gene activity. *In vivo* the phosphate groups are added and removed from the intact protein molecules in enzymatic reactions which are independent of protein synthesis and breakdown [3]. Results from enzymological and metabolic studies have led to the conclusion that the function of these phosphorylation and dephosphorylation reactions is to alter the conformation of these non-histone proteins, which in turn leads to changes in the structure and metabolic activity of the chromatin [12]. In support of this hypothesis, the quantity and phosphorylation rate of non-histone phosphoproteins has been found to be directly correlated with changes in chromatin structure and gene transcription in a variety of *in vivo* and *in vitro* situations [3–6, 13, 14].

It is clear from the present experiments that the non-histone chromatin phosphoproteins are heterogeneous and exhibit a tissue-specific pattern of distribution. Preliminary experiments on phosphoproteins prepared from rat liver have indicated that they are similar, but not identical, to phosphoproteins from bovine liver. Thus, in contrast to the histones, the non-histone phosphoproteins exhibit a pattern which varies between tissues and between organisms. Recent experiments have also shown that components of this non-histone protein fraction are capable of specifically recognizing and binding to certain types of DNA [15], thus further supporting the hypothesis that the non-histone chromatin protein play a key role in gene regulation.

Acknowledgements

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