EVIDENCE FOR THE PRESENCE OF NONHISTONE CHROMOSOMAL PROTEINS IN THE NUCLEOPLASM OF HeLa S_3 CELLS

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1. Introduction

The genome of eukaryotic cells is a nucleoprotein complex referred to as chromatin, consisting of DNA, RNA, and proteins. In differentiated cells at any given time the majority of the information contained within the DNA is repressed; and histones, or basic chromosomal proteins, have been implicated in this regard [1–5]. Recent results from several laboratories suggest that regulation of the transcriptional capacity of the genome [6–9], as well as the activation of specific regions of the genome [10–12], may be mediated by the nonhistone chromosomal proteins. These acidic proteins are tissue and species specific [13–20] and are present in relatively greater quantities in active than in inactive tissues [21] and in euchromatin as opposed to heterochromatin [22–27], and numerous fractions have a faster rate of turnover than histone [28]. Furthermore – unlike the histones, whose synthesis is restricted to the S phase of the cell cycle and tightly coupled with DNA replication [29–31] – the nonhistone chromosomal proteins are synthesized throughout the cell cycle [29, 32–38], independent of concomitant DNA synthesis [29, 39]. Evidence for nonhistone chromosomal proteins as regulators of gene expression has been summarized in several recent reviews [40–42].

Whether chromatin exists as a "fixed" material or whether the histones and nonhistone chromosomal proteins associated with DNA exchange between various intracellular compartments, has never been established. The present investigation attempted the partial resolution of this question by comparing the protein components of chromatin and nucleoplasm in exponentially growing HeLa S_3 cells.

2. Materials and methods

Exponentially growing HeLa S_3 cells maintained in suspension culture in Joklik-modified Eagle's Minimal Essential Medium (SM) supplemented with 3.5% each of calf and foetal calf serum were labeled at a concentration of 5×10^6 cells/ml in leucine-free SM containing 2% foetal calf serum and 2 μCi/ml L-[^{14}C]leucine. The cells were harvested by centrifugation, washed 4 times in cold Earle's Balanced Salt Solution, and nuclei were isolated by 4 washes with 60 vol of 80 mM NaCl, 20 mM EDTA, 1% Triton X-100. This procedure yields nuclei which are free of visible cytoplasmic material when examined by phase-contrast and electron microscopy [29]. The nuclei were washed in 0.15 M NaCl, 0.01 M Tris (pH 8.3), lysed in 0.001 M Tris (pH 8.3), and chromatin was pelleted by centrifugation at 12,000 *g* for 15 min. The supernatant constituted the nucleoplasmic fraction. Chromatin was further purified by centrifugation through 1.7 M sucrose.

3. Results and discussion

To establish that nucleoplasm prepared according to this procedure does not contain DNA, exponentially growing HeLa S_3 cells were incubated for 24 hr in [^{3}H]thymidine. Table 1 indicates that there is no significant [^{3}H]thymidine radioactivity associated with the nucleoplasmic fraction and that almost the total label resides with the chromatin.

Fig. 1 shows the distribution of L-[^{14}C]leucine radioactivity among the various chromosomal and nucleoplasmic polypeptide fractions resolved according

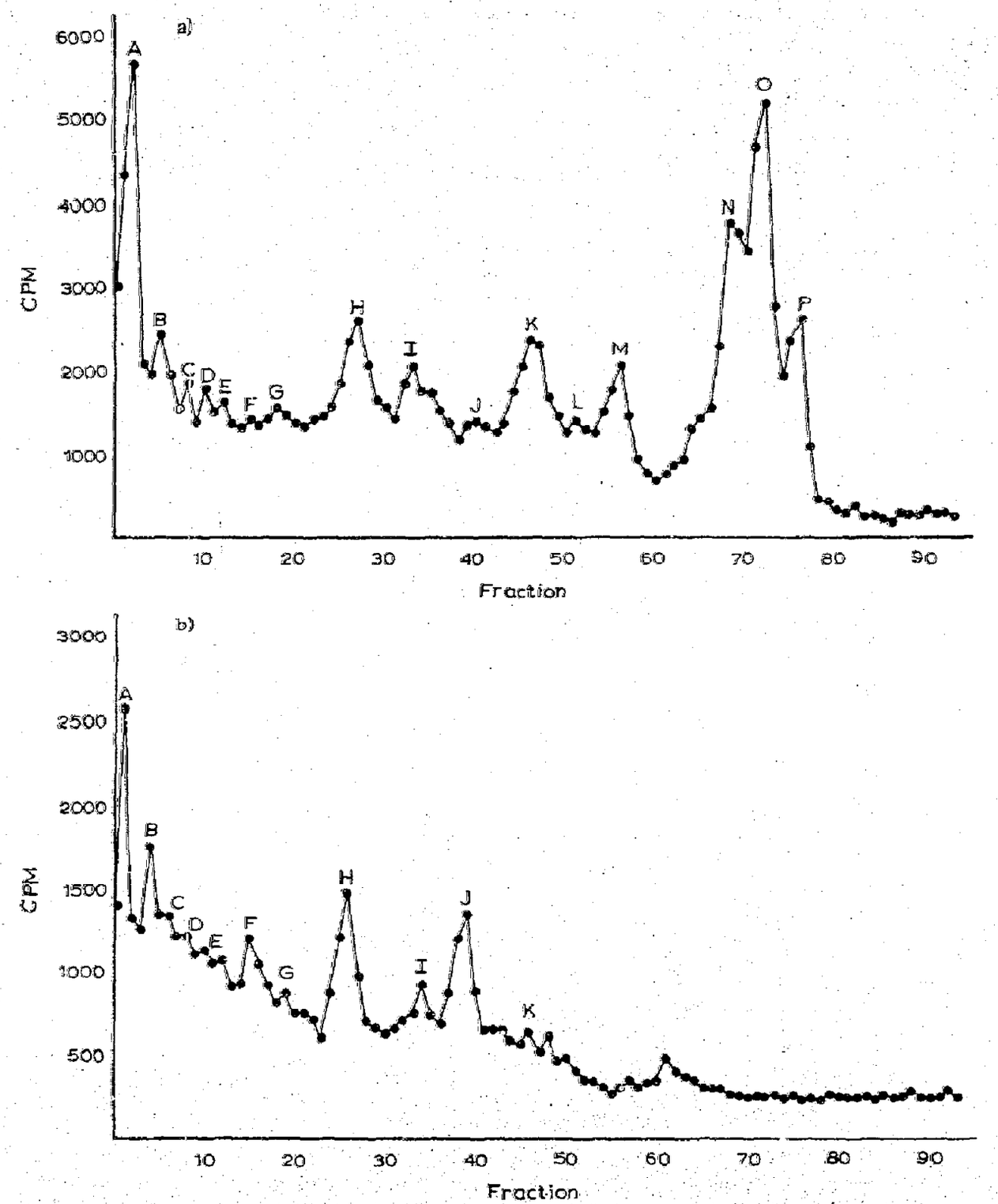

Fig. 1. a) SDS polyacrylamide gel electrophoretic profile of L-[^{14}C]leucine-labeled total chromosomal protein. Chromatin was solubilized in 1% SDS, 0.01 M phosphate, pH 7.0, and dialyzed for 12 hr at room temp. against 0.1% SDS, 0.01 M phosphate, pH 7.0. Electrophoresis of 0.25 ml samples containing 100 μg of proteins was carried out at room temp. for 11 hr at 90 V on 7.5% acrylamide gels, 0.6 × 20 cm [55]. The gel was fractionated mechanically, utilizing a Maizel Auto-Gel Divider [55], and collected in 100 vials, each containing 10 ml of Triton X-100-toluene cocktail [56]. Radioactivity was determined in a liquid-scintillation counter. The details of the procedure have been reported previously [29]. b) SDS polyacrylamide gel electrophoretic profiles of L-[^{14}C]leucine-labeled nucleoplasmic proteins. An equivalent amount of DNA (w/w) was added to the nucleoplasmic proteins, the samples were brought to a final conc. of 1% SDS, 0.01 M phosphate, pH 7.0, and subsequently dialyzed, electrophoresed, and fractionated as described in (a).

Table 1
Distribution of [^{3}H]thymidine radioactivity between chromatin and nucleoplasm.

Sample	(cpm/10^6 cells)
Total nuclei	48,795
Chromatin	46,796
Nucleoplasm	37

Exponentially growing cells were labeled for 24 hr with 0.1 μCi of [^{3}H]thymidine. Cells were harvested and nuclei were isolated and lysed and chromatin was pelleted as described in the text. The 10% trichloroacetic acid-precipitable radioactivity was determined from 100 μl aliquots of lysed nuclei (total nuclei), pelleted chromatin (chromatin), and the resulting supernatant (nucleoplasm).

to molecular weight on SDS polyacrylamide gels. There is evidence that in fractions 1–60, where the nonhistone chromosomal proteins migrate, several peaks are present in both the chromosomal and the nucleoplasmic proteins (peaks A–I). However, peak J is restricted to the nucleoplasm and peaks K, L, and M are found only associated with DNA as chromatin.

Evidence that nonhistone chromosomal proteins are confined to fractions 1–60 is provided by the distribution of L-[^{3}H]tryptophan radioactivity among the total chromosomal proteins fractionated on similar gels (fig. 2). Histones are present in these gels but lack tryptophan and are therefore not detectable. Fractions 61–95 represent the region of the gel where the histones migrate (determined by separately running purified histone standards on similar gels under these conditions), and a comparison of fig. 1a and 1b clearly demonstrates that these basic chromosomal proteins are absent in the nucleoplasmic preparation (peaks N, O, and P).

The banding patterns of chromosomal and nucleoplasmic proteins electrophoresed on SDS polyacrylamide gels and stained with Comassie Blue were compared [43]. Consistent with the radioactivity profiles in fig. 1, several protein peaks which migrate in the nonhistone chromosomal protein region of the gel are present in both the chromatin and the nucleoplasm. Furthermore, histone polypeptide bands are restricted to the chromatin.

These data suggest that, unlike the histones, which

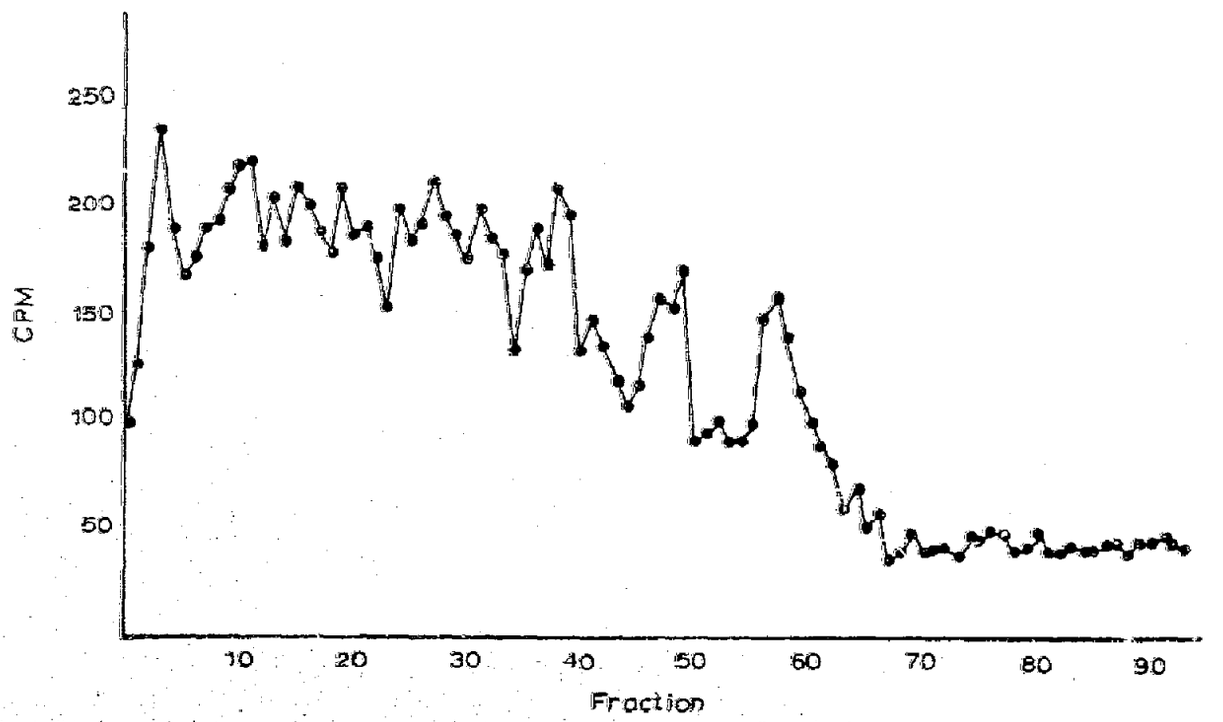

Fig. 2. SDS polyacrylamide gel electrophoretic profile of L-[^{3}H]tryptophan-labeled total chromosomal proteins. 2×10^8 HeLa S_3 cells were labeled for 30 min at a conc. of 5×10^6 cells/ml in tryptophan-free SM containing 2% foetal calf serum. Chromatin was prepared as described previously in the text, solubilized in 1% SDS, 0.01 M phosphate, pH 7.0, dialyzed against 0.1% SDS, 0.01 M phosphate, pH 7.0, and electrophoresed as described in fig. 1a.

are found only in association with DNA, several species of nonhistone chromosomal proteins are present in the chromatin as well as in the nucleoplasm. Such findings – taken together with the tissue and species specificity of nonhistone chromosomal proteins [13–20], differences in the classes of nonhistone chromosomal proteins synthesized during various phases of the cell life cycle [29, 32–38] and when a given cell is induced or committed to a unique differentiated function [44–53], as well as the influence of these proteins on selective transcription of chromatin [6–12] – are consistent with the concept of nonhistone chromosomal proteins as regulators of gene expression [40–42]. Yet the specific manner in which nonhistone chromosomal proteins interact with the genome to mediate the transcription of informational macromolecules is at present unknown.

It is, however, reasonable to speculate that chromatin is not a "fixed" material, but rather, that is exists in a "semifluid" state; and the model which we propose to account for the genome of eukaryotic cells contains two classes of macromolecules which constitute "static" and "fluid" components. Nuclear DNA and histones represent the "static" components, since these chromosomal constituents exhibit negligible rates of turnover [54] and reside solely within the nuclei complexed with one another. Certain classes of nonhistone chromosomal proteins which are metabolically stable, lack tissue or species specificity, and are found only associated with DNA, may also be included in this category. The "fluid" component of chromatin may be represented by those nonhistone chromosomal proteins which possess specificity and rapid rates of turnover. Perhaps it is these macromolecules which are involved in the regulation of DNA-dependent RNA synthesis and exist in equilibrium with a nucleoplasmic pool. Studies are presently being directed towards determining whether, in fact, these nonhistone chromosomal proteins which are present in both chromatin and nuclear sap are responsible for the control of gene expression.

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