LOW LEVELS OF NON-HISTONE CHROMOSOMAL PROTEINS IN TROUT TESTIS CHROMATIN

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1. Introduction

In studies on the regulation of gene expression, there has been considerable interest in the non-histone (NHC) proteins associated with histones and DNA in chromatin [1–5]. Such NHC proteins are reportedly present in large amounts, i.e. 50–150% of DNA content by weight [1,2], and display some tissue and species specificity [1–5], which has implicated them in gene activation and regulation [1–8]. Other NHC proteins would have structural or enzymatic functions in chromatin.

However, it is evident that in the isolation of NHC proteins: (i) structural rather than regulatory proteins would likely be detected [3,5,14]; and (ii) of these putative structural NHC proteins, some may be artifacts of cytoplasmic [9], membrane [10,19] or other [20] contamination.

For trout testis, the large ratio of nuclear to cytoplasmic volume makes it relatively easy to prepare 'clean' chromatin and examine its NHC proteins.

We wish to report that NHC proteins are present at less than 5% of DNA content by weight. This result holds even for early testis, where DNA, RNA and protein synthesis, as well as cell division, are still ongoing processes [11], and the structure [12] and function of chromatin are similar to those of other tissues. The same observation has been made in calf thymus [13], another tissue from which chromatin is readily purified.

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Such results suggest caution is necessary in assigning to any protein a chromatin localization, with accompanying regulatory or structural roles. Some NHC proteins may instead be contaminants from other cellular locations.

2. Materials and methods

2.1. *Preparation of trout testis nuclei and chromatin*

Nuclei and chromatin were prepared as previously described [12]. In some experiments, nuclei were also washed with buffer containing 0.1% of the non-ionic detergent NP40 (Shell) to remove potential membrane contamination. Naturally maturing trout testes were obtained at stages corresponding to 4–5 weeks and 6–7 weeks of hormonal induction, i.e. early histone and histone stage [22].

2.2. *Preparation of NHC proteins*

Several different methods were tried.

Method A: Chromatin was sequentially treated with salt, acid, buffer-saturated phenol (pH 8.2), and hot sodium dodecyl sulfate (SDS) as described by LeStourgeon and Rusch [14].

Method B: Chromatin was extracted with 0.4 N H_2SO_4, organic solvents and finally 1% SDS, 0.14 M 2-mercaptoethanol, essentially as described by Elgin and Bonner [15].

Method C: Chromatin was treated with DNase I (Worthington) and then directly extracted with hot SDS (5–10%).

Method D: Chromatin was extracted with 0.4 M NaCl [13], and proteins in the resultant extract were concentrated by ammonium sulfate precipitation (70% saturation).

Proteins were quantitated by the Lowry method [16], or by electrophoresis on 5% SDS polyacrylamide gels [17], while DNA was quantitated by its absorbance at 280 nm or by the diphenylamine reaction [18].

3. Results and discussion

Employing methods A, B, C or D, we were unable to isolate NHC proteins in amounts exceeding 5% by weight of DNA. A scan of total chromosomal proteins (Method D) shows very little protein other than histones and the added DNase I (fig.1a). After concentration of 0.4 M salt-dissociated proteins (Method D) by $(NH_4)_2SO_4$ precipitation, a number of bands are evident (fig.1b), but in very small amounts relative to histone and DNA content. It is also apparent that a major proportion of the protein in 1b migrates in positions corresponding to histones. There is, however, one major component (X) which predominates in pattern 1b, and can also be seen in whole chromatin (1a). Its molecular weight is approx. 140 000. Other proteins can be removed from acid-extracted histones by carboxymethyl cellulose chromatography, but these are present in extremely low yield, and some co-electrophorese with trout ribosomal proteins on starch gels. (B. M. Honda, unpublished results).

Previous reports have indicated that chromatin can be contaminated by cytoplasmic [9], membrane [10,19] or other nuclear components [20] leading to the artifactual isolation of NHC proteins. Even attempts to radioactively label regulatory NHC proteins in chromatin may suffer from the same limitations, as noted by Harlow and Wells [19] and Pederson [20].

The low levels of NHC proteins in trout testis and calf thymus [13] further the idea that in other tissues studied it may be difficult to obtain purified chromatin and NHC proteins. It is possible that trout testis and calf thymus are 'unusual' tissues; however, testis cells are still actively dividing and carrying on DNA, RNA and protein synthesis in the early stages used in these experiments. It is also conceivable that certain specialized chromosomes e.g. *Drosophila* polytene chromosomes, may require special structural and NHC proteins [3].

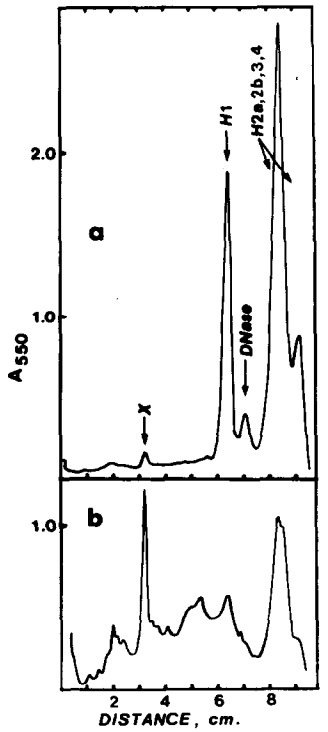

Fig.1(a) Total proteins extracted from trout testis chromatin. Nuclei were prepared as described [12] and washed with 50 mM Tris–HCl pH 8.0 containing 1 mM $MgCl_2$, 25 mM KCl and 1% Nonidet P40. After centrifugation (3000 *g*, 10 min) the pellet, corresponding to 2 g of testis, was resuspended in 1.0 ml of 0.1 M sodium acetate pH 5.0 containing 10 mM magnesium acetate, and digested with 100 μg of DNase I (Worthington) for 2 h at 22°C. The sample was then dialyzed against 60 mM NH_4HCO_3 and lyophilized. The lyophilized extract was then redissolved in 10% SDS-0.14 M 2-mercaptoethanol and heated for 2 min at 100°C. An aliquot of this sample (0.25% of total) was then analyzed on a 5% SDS-PO_4 acrylamide gel [17]. Gels were stained with Coomassie Blue, destained and scanned at 550 nm. (b). Proteins extractable from chromatin at low salt concentrations. Nuclei, prepared and washed as in 1a above were treated with 0.4 M NaCl, and centrifuged at 10 000 *g* for 20 min. The supernatant was adjusted to 70% saturation with ammonium sulfate, and the protein precipitate was collected by centrifugation at 10 000 *g* for 20 min. The pellet was redissolved in 0.1 M NH_4HCO_3, dialyzed against the same buffer, and lyophilized. The lyophilized protein was then treated with SDS-mercaptoethanol and analyzed as described in (a). The amount of chromatin used for gel 1b was 1/8 of that used in (a).

The nature of the NHC proteins isolated from trout testis is unclear. After the finding that chromatin subunits (nu bodies) from trout testis contained negligible amounts of NHC proteins [12], it was thought that NHC proteins might be bound to the 'open' DNA not protected in subunits. However, the present results indicate that whole chromatin from testis is also low in NHC proteins. Some of these NHC proteins probably represent structural [14], contractile [21] and enzymatic components of testis chromatin while others may still be contaminants.

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