

Histones of Nucleolar-associated Chromatin from Hamster Liver

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The nucleolus, a distinct biochemical and morphological entity, is the site where precursor ribosomal RNA is synthesized, processed to mature rRNA and assembled with proteins to preribosomal particles (Miller 1981; Busch 1982; Sommerville 1985, 1986). Considerable interest developed on the functions of nucleolar proteins in ribosome biosynthesis. Two major nucleolar phosphoproteins, designated as B23 (mol. wt/pI = 38 000/4.9—5.4) and C23 (mol. wt/pI = 110 000/5.5) were purified and characterized (Olson 1983). It was suggested that the protein C23 may play a role in the rRNA gene regulation. The protein B23 is probably involved in the process of maturation of ribosomal structures (Spector et al. 1984). Moreover, it is well established that histone fractions are basic protein components of nucleolar chromatin (Busch and Smetana 1970; Olson and Busch 1978). However, it was presumed that transcribed rDNA is devoid of histones and nucleosomes and that its behaviour resembles that of free DNA (Franke et al. 1979; Labhart et al. 1983). Hence, it can be assumed that histones are mainly located in the inactive, nontranscribed form of nucleolar chromatin. The results of a series of experiments from Gurley's laboratory (Halleck and Gurley 1980, 1982) on cultured cell lines of the deer mouse *Peromyscus* suggest that constitutive heterochromatin is enriched in a more hydrophobic variant of the histone H2A, i.e., H2A.1. The histone complement of hamster liver nuclei was characterized electrophoretically in our previous work (Kiliańska et al. 1982)). In the present work the histone patterns of hamster liver nucleoli and nuclei were compared by electrophoresis in acid-urea and acid-urea-Triton X-100 gels. The latter electrophoretical system is a very valuable tool for the analysis of histone microheterogeneities and hydrophobic properties.

Nucleoli from hamster liver. Nuclei from male Syrian hamster liver were obtained by the sucrose method with additional treatment of nuclei with 0.4% Triton X-100 to remove membrane ghosts. They were purified by centrifugation at $40,000 \times g$ through 2.2 mol/l sucrose. The nuclear pellet was resuspended

(1 mg DNA/ml) in 0.25 mol/l sucrose, 1 mmol/l CaCl_2 , 5 mmol/l Tris . HCl (pH 7.4), sonicated for 15 s in 30 s intervals until no nuclei remained intact, and centrifuged through 0.9 mol/l sucrose, 10 mmol/l Tris . HCl (pH 7.4) at $1600 \times g$ for 20 min. The crude nucleolar pellet was resuspended in 0.25 mol/l sucrose, 1 mmol/l CaCl_2 , 5 mmol/l Tris . HCl (pH 7.4) and resonicated to disperse the extranucleolar chromatin. The sonicate was centrifuged through 0.9 mol/l sucrose, 10 mmol/l Tris . HCl (pH 7.4) at $1600 \times g$ for 20 min. The nucleolar pellet was washed a few times in the same manner in low calcium-sucrose buffer (Busch 1967).

Nuclear and nucleolar extractions. Nuclei and nucleoli of hamster liver were first washed with the following solutions: 0.14 mol/l NaCl, 20 mmol/l sodium citrate; 10 mmol/l Tris . HCl buffer (pH 7.2) and 0.34 mol/l NaCl. Subsequently total histone was extracted with 0.25 mol/l HCl. The protease inhibitor, phenylmethanesulfonyl fluoride (PMSF) was added fresh to all solutions used in this study to a final concentration of 1 mmol/l.

Polyacrylamide gel electrophoresis. Analytical gel electrophoresis of histones was performed in two different systems: (i) in acid-urea-15% polyacrylamide gels according to Panyim and Chalkley (1969), (ii) in acid-urea-Triton X-100-12% polyacrylamide gels according to Zweidler (1978). The gels were scanned at 560 nm using an ERJ 65 densitograph (Carl Zeiss). The proportions of histone subfractions were determined by integration of the areas beneath the peaks on the densitometric tracings.

The results of analytical electrophoresis of the histones from nucleoli of hamster hepatocytes performed in the acid-urea polyacrylamide gels according to the Panyim and Chalkley's method (1969) demonstrate that the nucleolar-associated chromatin as well as nuclear chromatin contain five major histone fractions (Fig. 1i).

The electrophoretic resolution of the histones can be significantly improved over the acid-urea system by the addition of Triton X-100 to polyacrylamide gels. Using the optimum concentration of the urea (7.5 mol/l) and Triton X-100 (6 mmol/l) it became possible to resolve H2A, H2B and H3 into their primary structure variants as well as phosphorylated and acetylated forms of these histones (Franklin and Zweidler 1977; Zweidler 1978; Urban and Zweidler 1983). The nucleolar histone components of hamster liver resolved by Triton X-100 polyacrylamide gel electrophoresis behaved similarly to those of nuclei (Fig. 1ii). When the densitometric tracings of the electrophoretic patterns of histones from nucleoli and nuclei of hamster hepatocytes were compared no differences in the relative proportions of the histone H2A variants, i.e., H2A.1 and H2A.2 were found. However, differences in the relative proportions of

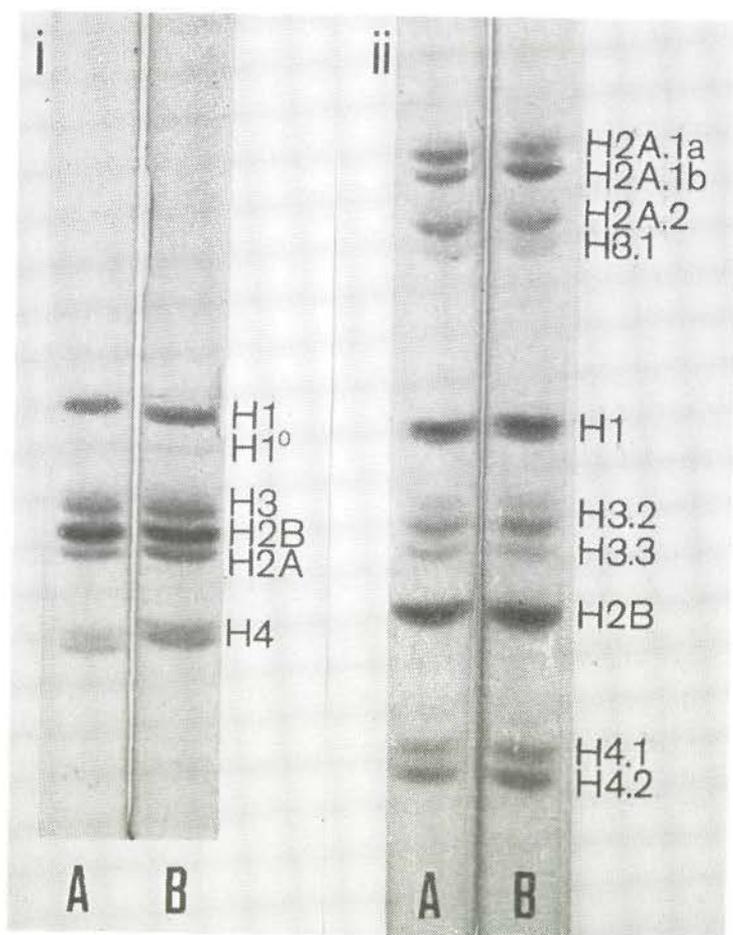


Fig. 1. Electrophoretic patterns of total histone from hamster liver nuclei (*A*) and nucleoli (*B*) after electrophoresis performed in: (i) 15% polyacrylamide gels containing 2.5 mol/l urea (pH 2.7) according to Panyim and Chalkley (1969), (ii) 12% polyacrylamide gels containing 7.5 mol/l urea and 6 mmol/l Triton X-100 according to Zweidler (1978). Gels were stained with Naphthalene Black 10B. (A typical picture of 3 independent analyses).

phosphorylated forms of histone H2A.1 were noted. Table 1 shows that the phosphorylated form of the histone H2A.1 designated H2A.1a from nucleoli and nuclei constituted about 28% and 40% of the total histone H2A, respectively, and the subfractions H2A.1b from nucleoli and nuclei made up 43% and 30%, respectively. The histone H2A.2 from nucleoli and nuclei of hamster liver amounts to about 28% of the total histone H2A. These results suggest that

Table 1. The relative content of the histone H2A variants from nucleoli and nuclei of hamster liver. The mean values \pm SD of the amount of each variant from three measurements for three separate preparations are given as the percentages of total histone H2A

	Hamster liver	
	Nucleoli	Nuclei
H2A.1a	28.4 \pm 1.7	40.7 \pm 1.7
H2A.1b	43.7 \pm 1.6	30.3 \pm 2.9
H2A.2	28.0 \pm 2.7	29.0 \pm 1.1

nucleolar-associated chromatin is reduced in a phosphorylated form of the histone H2A.1 when compared with nuclear chromatin. The functional implications of these observations are not clear.

Allis et al. (1982) stated that a protein antigenically related to a minor variant of H2A, hv 1 in the *Tetrahymena macronucleus* is enriched in nucleolar chromatin of mammalian cells. Bhatnagar et al. (1984) reported also qualitative changes in the histone composition of somatic mammalian nucleolar chromatin. The data presented show that nucleolar-associated chromatin of liver and brain nuclei of the mouse is highly enriched in protein "A", an H2A variant which probably corresponds to H2A.X.

It seems likely that these changes can be associated with the unusual structure of nucleolar chromatin.

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