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HISTONES IN DIFFERENT CHROMATIN FRACTIONS FROM PIG LIVER AND IMMATURE PIG TESTIS[1,2]

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ABSTRACT

T. C. Lee and K. Y. Jan (1977). *Histones in Different Chromatin Fractions from Pig Liver and Immature Pig Testis.* Bull. Inst. Zool., Academia Sinica **16**(1): 57-61. The electrophoretic pattern of histones from pig liver nuclei and immature pig testis nuclei were similar to that from the calf thymus, but minor differences were observed on H1 and H4. Heterochromatin and euchromatin from pig liver and immature testis were separated by differential centrifugation. The heterochromatin contains slightly more histones than the euchromatin, but the electrophoretic pattern of their histones were remarkably similar. Therefore the histones may not play an important role in chromatin condensation or in the C band formation on metaphase chromosomes.

It is well known that in most eukaryotic cells, the nucleus is composed of heterochromatin, condensed regions devoid of transcriptional activity, and euchromatin, extended regions which include the transcriptionally active genes. Yasmineh and Yunis[25] claimed that heterochromatin and euchromatin can be separated by differential centrifugation. The separation of heterochromatin and euchromatin should allow a determination of the biochemical components responsible for their transcriptional activities and their morphological features under the microscope. The role of histones in maintaining the structure and regulating the function of chromatin has long been suggested. Indeed, both of these roles may be closely correlated, the inhibition of RNA synthesis being a consequence of the histone-induced superhelical structure of DNA. In this communication we report the quantitative and qualitative analyses of histones in hetero- and euchromatin.

MATERIALS AND METHODS

Freshly isolated livers from mature pigs and testis from pigs of about 4 weeks old were quickly frozen by liquid nitrogen or dry ice in acetone. High purity of nuclei from these tissues were prepared by the procedures of Chauveau *et al.*[6]. Their chromatin were frac-

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tionated into 3 portions by differential centrifugation technique of Yasmineh and Yunis[25]. Accordingly, the pellets of 3,500 xg was designated as chromatin fraction 1, pellets of 78,000 xg as fraction 2 and the supernatant as fraction 3. Fraction 1 was refered as heterochromatin and fractions 2 and 3 as euchromatin. For UV[7] spectrum analysis, the chromatin concentrations were adjusted to the final O. D. of 1.0 at 360 nm in 0.01 M tris buffer (pH 8.0) and scanned by a Hitachi 124 spectrophotometer.

After the removal of RNA by the incubation in 0.3 N KOH at 37°C for 18 hrs, the DNA content in each chromatin fraction was estimated by the method of Webb and Levy[23] using calf thymus DNA as a standard. Histones in purified nuclei or alcohol precipitated chromatin were extracted twice by 0.4 N sulfuric acid for 1 hr each precipitated in alcohol, washed twice in acetone and finally dried in vacuum. The protein content in each chromatin fraction was estimated by the method of Lowry *et al.*[13] using calf thymus histones and bovine serum albumin as a standard. The histones from purified nuclei and fractionated chromatin were analysed by polyacrylamide gel electrophoresis as described by Panyim and Chalkley[18]. The gels were traced by ISCO gel scanner at the wave length of 580 nm.

RESULTS AND DISCUSSION

The UV spectrum is one of the criteria for quick determination of the purity of chromatin. The chromatin curves on Fig. 1 are similar to those reported by many workers[2,3,10]. In Fig. 1, the order of the absorption between 230–260 nm is L1>L2>L3 (chromatin fractions from liver) and T2>T1>T3 (chromatin fractions from testis). This same order is also observed on the nonhistone content as indicated on Table 1. On the other hand, the order of histone content is L1>L2>L3 and T1>T2>T3, but the differences are much less prominent. These results are comparable[7] to those reported by Burkholder and Weaver[5].

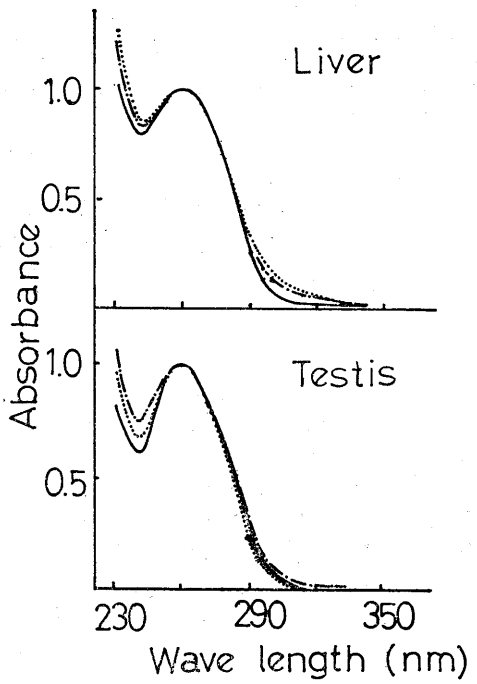

Fig. 1. Asorption spectra of fractionated chromatin from pig liver and immature pig testis, ··· fraction 1, -·-·- fraction 2, and — fraction 3.

Whereas, chromatin fractionated by DNase II digestion combining with 0.15 M sodium citrate solubilities has shown that template-inactive portion was relatively rich in histones but poor in nonhistones[14]. The relative poor in histones and rich in nonhistones of template-active chromatin were also reported by Berkowitz and Doty[1]. Although the relative high histone content in heterochromatin are consistent, but the high nonhistone content in heterochromatin as separated by differential centrifugation technique is not understood. It should be mentioned that many workers[7,8,17,22] have indicated that a significant amount of nonhistones were extracted with 0.2 N HCl or 0.4 N H_2SO_4. Therefore, the amount of acid soluble versus acid insoluble proteins may not represent the actual amount of histones and nonhistones.

TABLE 1.

The ratios of histones and nonhistones to DNA in different chromatin fractions

		Chromatin fractions		
		1	2	3
Histones	Liver	0.88±0.01	0.86±0.02	0.79±0.01
	Testis	0.91±0.01	0.89±0.04	0.76±0.03
Nonhistones	Liver	1.70±0.13	1.40±0.07	0.93±0.01
	Testis	1.59±0.05	2.12±0.25	1.15±0.03

In Fig. 2 the H3, H2b and H2a of calf thymus, pig liver and immature pig testis were almost identical, but minor differences were observed in H1 and H4. Thus, the present data are in agreement with the previous demonstration that histones are relatively poor in organism and tissue specificities[21]. The H1 of both pig liver and immature testis consisted of 4 bands, but the H1 of pig liver migrated as 1+3 distribution and the H1 of immature testis migrated as 2+2 distribution, whereas the H1 of calf thymus consisted 3 bands and migrated as 3+0. The H4 of calf thymus, pig liver and immature testis all consisted of 2 bands, but the 2 bands of pig liver H4 were slightly apart from each other. The finding that H1 variations in the pig liver and testis is not uncommon, since the H1 has been shown to be a family of related protein components that exhibit considerable tissue specific variation[15,19,22]. In sea urchin the complexity of H1 increases from a single species in morulae to three species in gastrulae[21].

The electrophoretic pattern of histones from nuclei (Fig. 2) were slightly different from that of fractionated chromatin (Fig. 3). On the other hand, no apparent difference was

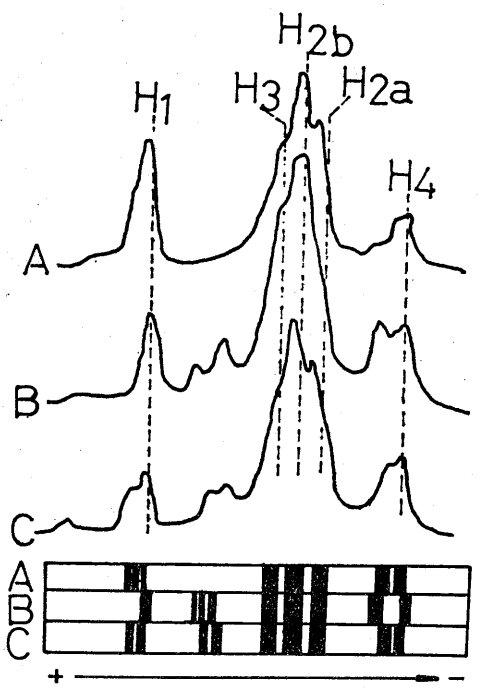

Fig. 2. Electrophoretic pattern of histones from A. calf thymus, B. pig liver nuclei and C. immature pig testis nuclei.

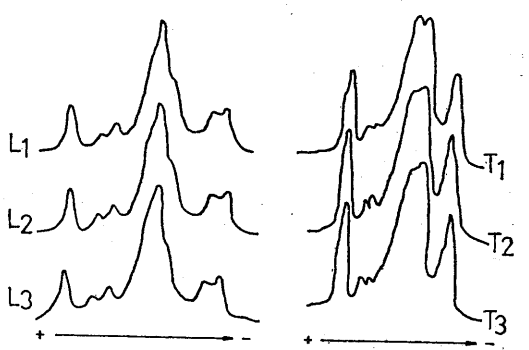

Fig. 3. Electrophoretic pattern of histones extracted from different chromatin fractions.

found among the banding pattern of histones from L1, L2 and L3, nor from T1, T2 and T3 (Fig. 3), except the proportion of H2b to H2a of T1 seems slightly different from that of T2 and T3. Since the euchromatin of L2, L3 and T2, T3 are not all engaged in transcription, these results are not necessarily against the recent finding of Berkowitz and Doty[1] in which the H1, H5 and H3 were absent from the transcription active chromatin.

Remarkable similarity of histones between condensed and extended chromatin were also found in mouse liver[12]. Furthermore, the heterochromatin distribution in avian erythrocytes and liver nuclei were unchanged after complete histone extraction[4]. These data seem to suggest that histones may not function in chromatin condensation. Inasmuch as constitutive heterochromatin on metaphase chromosomes can be located by C-band technique[20], the present results strongly indicate that the histones may not participate in C bands formation. This view is consistent with a previous finding that C bands was not affected by the extraction of histones[11]. The general similarities in electrophoretic mobilities[19] and relative conservation of amino acid sequence of histones[9] all point to the possible implication that in all eukaryotic chromosomes, the DNA may be packed in a similar manner, for example the nu body as observed by several workers[16,24].

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不同染色質間鹼性核蛋白之比較分析

李 德 章　詹 崑 源

由豬肝細胞核及乳豬睾丸細胞核所萃取之 鹼性核蛋白其電泳帶分佈與來自 牛胸腺之鹼性核蛋白大致相似，但在 H1 及 H4 處略有不同。用不同離心力將豬肝及乳豬睾丸之染色質劃分成異染色質及眞染色質，發現鹼性核蛋白之含量在異染色質中稍多，但鹼性核蛋白電泳帶之分佈在異染色質與眞染色質之間並無明顯之差異。因此推論鹼性核蛋白對於染色質之緊縮或 C 染色帶之形成可能無重要影響力。