Age-related changes of the pattern of non-histone proteins in active and condensed fractions of mouse liver chromatin and hepatocarcinoma

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Summary. Electrophoretic analysis of histones and non-histone acid-soluble proteins in active (nuclease sensitive) and inactive chromatin from liver of young and old CBA mice and in age-related hepatocarcinomas showed a higher ratio of NHP: histones in active chromatin in old cells. Some liver- and hepatoma-specific fractions of non-histone proteins have been identified as chromatin matrix proteins.

Key words. Liver, mouse; aging; nonhistone proteins; chromatin; hepatoma.

The tissue specificity of the pattern of non-histone chromatin proteins (NHP)[1-3] apparently reflects their role in the regulation of tissue-specific transcription of genetic information. This has made it logical to make some attempt to find cancer-specific chromatin proteins as well. Several minor novel nuclear and chromatin NHP had been identified by biochemical and immunological methods in chemically-induced or transplantable hepatomas in rat and mouse[4-8]. However, age-related spontaneous hepatocarcinomas have not yet been studied in the same way, despite the fact that most forms of cancer are related to some age changes of cells and have an increased incidence during ageing. We recently described 2 liver-specific acid-soluble NHP (LSP1 and LSP2) which showed a significant age-related increase in rat liver chromatin[9]. The amounts of the same proteins did not show a visible increase in normal liver in aged CBA mice, but showed a sharp (5- to 6-fold) increase in age-related spontaneous hepatocarcinomas[10]. Because CBA mice are a long-lived strains specially known for their high incidence of hepatocellular neoplasms (up to 40–60% in aged animals)[11,12], it was interesting to study the chromatin localization of NHP which in the case of the rat are relevant for age changes of liver chromatin, while in the case of CBA mice they are connected with the malignant transformation of liver cells.

Materials and methods. The livers were removed from Mill Hill CBA strain mice, frozen and stored at −80 °C until required for analysis. 40 livers from young (3–4 months old) and old (28–29 months old) mice were used to isolate nuclei in each experiment. In every group of old CBA mice, about 20–30% of livers have easily recognizable, solid hepatocarcinomas of different size. They were excised and collected separately. Nuclei were isolated by the method of Schibler and Weber[13]. Chromatin was obtained by repeatedly blending nuclei in a Dounce homogenizer with 0.025 M EDTA in 0.07 M NaCl, pH 7.5. All loosely bound NHP, including the High Mobility Group proteins, were removed by 0.35 M NaCl extractions of chromatin. The remaining chromatin was divided into active and inactive fractions by the method of Tata and Baker[14], which is based on the higher sensitivity of DNA in active chromatin to a mild digestion with micrococcal nuclease (MCase). The method was modified in our case in order to make additional, longer digestions of inactive chromatin (fractions S2 and S3). The first digestion (0.8 units of enzyme per ml for 90 sec at 29 °C) destroys about 12–15% of DNA which is en-

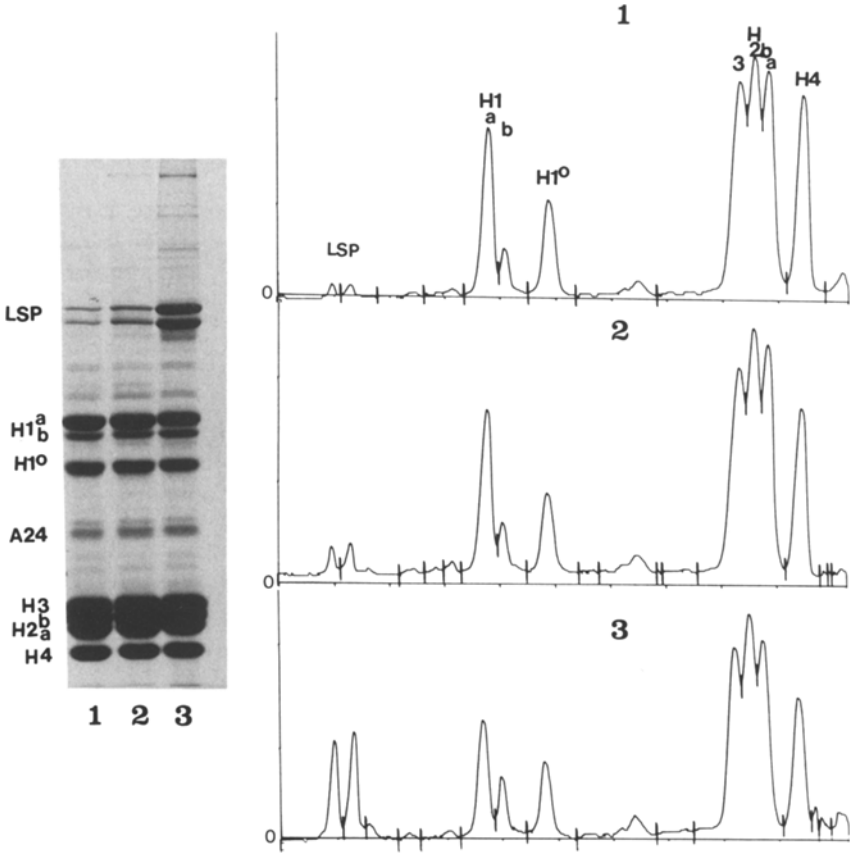

Figure 1. SDS-PAGE profiles of histones and liver-specific non-histone proteins (LSP) extracted with 0.25 M HCl from liver chromatin. 1. Normal livers of 28-month-old mice. 2. Sections of livers separated from solid spontaneous hepatocarcinomas. 3. Spontaneous age-related hepatocarcinomas.

riched in active genes and releases mononucleosomes. The 2nd and 3rd digestions of the remaining inactive Pl chromatin were carried out with a high concentration of nuclease (1.6 units/ml and 8 units/ml and for longer times, 4 and 5 min, respectively). After 3 consecutive digestions about 60% of chromatin DNA had been destroyed. Supernatant fractions and the pellet were extracted with 0.25 M HCl to isolate histones and acid soluble NHP. Purification of extracted proteins and electrophoresis was carried out as described earlier[10]. The amount of DNA in different fractions was determined by measuring the absorbance of the DNA hydrolysate (15 min at 70 °C in 7% perchloric acid) at 260 nm. The DNA-binding capabilities of chromatin proteins were studied by the method of Bowen et al.[15].

Results and discussion. The sharp increase of liver-specific NHP in spontaneous hepatocarcinomas in 28-month-old mice is shown in figure 1. The amount of LSP present in hepatoma chromatin is so high that it is almost comparable with the amount of Hl or any other histone. This makes LSP1 and LSP2 in hepatomas the most prominent non-histone proteins in chromatin compared with any NHP described earlier in any type of tissue. It is, therefore, important to establish the possible localization of LSP in chromatin. The pattern of histones and other 0.25 M HCl-soluble proteins in 'active' and 'inactive' fractions of chromatin isolated from livers of young and old mice is shown in figure 2. The amount of sample loaded for each lane was adjusted to have equal amounts of core nucleosomal histones in the NHP:histones ratios in different fractions. It can be seen that the ratio NHP:histones is highest in active chromatin from livers of old animals. There are some differences of the pattern related to ageing as well (some prominent age-specific bands marked as X in the area of molecular weight of 30,000–40,000). This does not mean there is an increase in the amount of all NHP, some are known to be lower in older tissues which have higher proportion of inactivated condensed chromatin[16–18]. In our case, only acid-soluble NHP which were more tightly bound in chromatin were studied. The larger part of NHP was extracted by 0.35 M NaCl before the MCase digestion. At the same time 2 liver-specific NHP are present only in 'inactive' chromatin which remains in the pellet after repeated digestion with MCase (P2 and P3) when about 50% of total DNA is digested. This makes it possible to suggest that LSP1 and LSP2 belong to a group of so-called chromatin matrix proteins. The experiment which shows that LSP1 and LSP2 are not DNA binding proteins (fig. 3) is also consistent with this suggestion. Chromatin matrix proteins have a certain influence on transcription through their role in chromatin general structure[19]. It is possible that the unusually high amounts of novel matrix proteins in ageing liver cells may lead to the carcinogenic transformation in CBA mice strain which is genetically predisposed to produce hepatocellular neoplasm. It was also interesting to find a visible increase of more tightly bound NHP in 'active' parts of chromatin in old animals. There were some theoretical suggestions about the possible increase of tightly bound chromatin proteins during cellular aging[20,21] but they were based on the analysis of the denaturation pattern ('melting profile') of chromatin, not on direct study of the pattern or localization of tightly bound chromatin proteins.

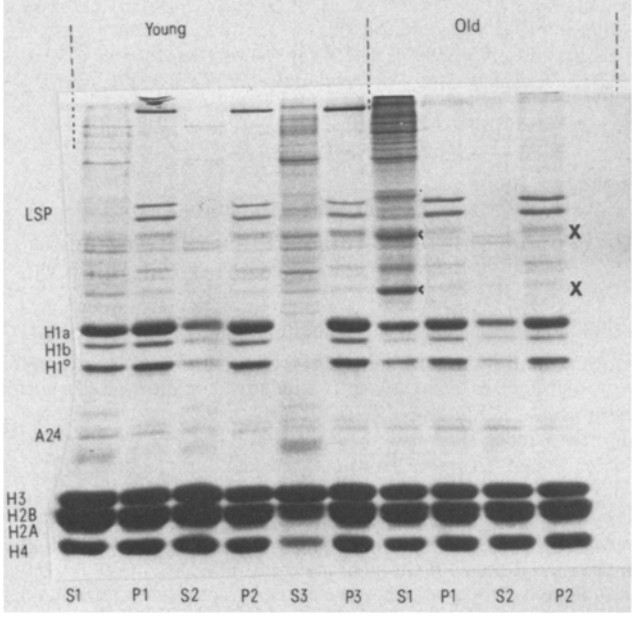

Figure 2. SDS-PAGE pattern of histones and 0.25 M HCl soluble non-histone proteins extracted from different fractions of liver chromatin from young and old mice. S1: Histones and NHP extracted from supernatant ('active') fraction after short (90 sec) digestion with micrococcal nuclease. P1: Histones and NHP extracted from undigested pellet ('inactive') chromatin. S2: Histones and NHP extracted from supernatant chromatin fraction released after additional treatment of inactive Pl pellet with micrococcal nuclease. P2: Histones and NHP extracted from residual pellet after 2 nuclease digestions. S3: Histones and NHP extracted from supernatant chromatin fraction released after treatment of P2 fraction with increased concentration of micrococcal nuclease (see materials and methods). P3: Histones and NHP from remaining undigested pellet. The quantity of sample loaded on the gel was adjusted to have equal amounts of nucleosomal core histones (about 20 µg in each lane). The picture does not reflect the ratio proteins: DNA and nucleotides in digested fractions. This ratio is usually high in S1, but rather low in S2 and very low in S3.

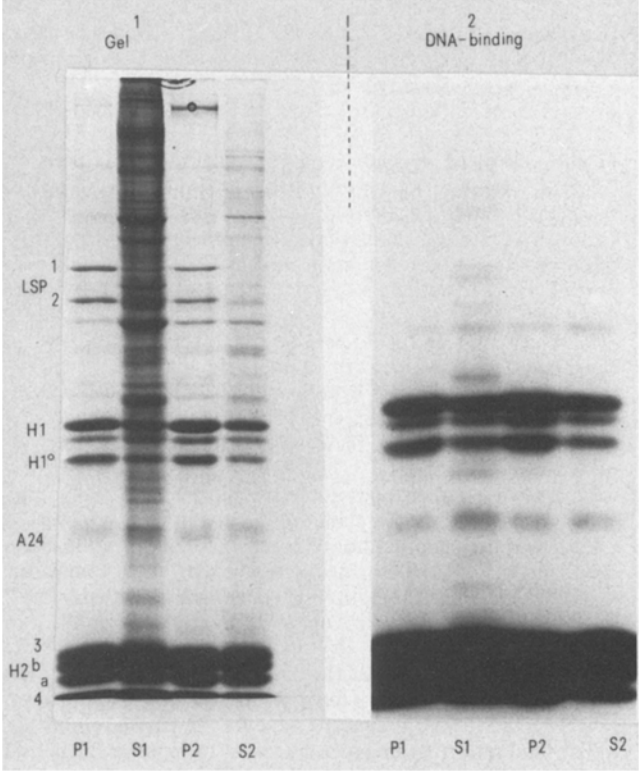

Figure 3. 1. SDS-PAGE pattern of 0.25 M HCl soluble proteins from liver chromatin of 28-month-old CBA mice after treatment with micrococcal nuclease. P1: Non-digested pellet after mild digestion (0.8 U/ml for 90 sec at 29 °C); S1: Proteins from supernatnant ('active') fraction of chromatin; P2: proteins from pellet precipitated after second longer digestion of P1 ('inactive' chromatin); S2: Supernatant fraction after second digestion. 2. [^{32}P]DNA binding to proteins transferred by protein blotting from the duplicate of gel 1 (unstained) into nitrocellulose filters.

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Dark exposure inhibits the mitotic activity of thyroid follicular cells in male mice with intact pineal

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Key words. Mice, male; dark exposure; activation, light-restriction-induced; pineal; thyroid follicular cells, mitotic activity.

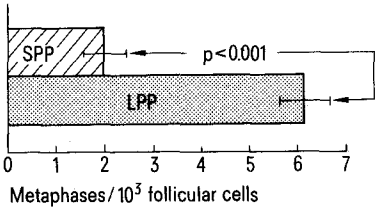
Pineal control of thyroid secretion and growth has been recently a subject of intensive research. An inhibitory effect of the pineal gland on thyroid function has been suggested[2]. However, the site of inhibition of hypothalamo-pituitary-thyroid axis has not been determined precisely, although data supporting both hypothalamic and peripheral direct effects are available[3,4].

Studies dealing with the influence of the pineal on the histological appearance and growth of the thyroid gland, primarily relating to the indices of hypertrophy, have produced contradictory results. Following pinealectomy (PX), enlargement of the thyroid, resulting in increase of its weight, has been reported by many authors[4–9]; however, negative reports also exist[10–12]. Histologial changes in the thyroid after PX, suggesting increased activity of the gland and similar to those observed after thyroid stimulating hormone (TSH) administration, have been reported[8,9]. Other investigators have not found the marked histological alterations in the thyroid following PX[4]; others even have shown changes which would be unlikely to accompany the increased activity of the thyroid[3].

Until now, the influence of the pineal gland on thyroid hyperplasia has been examined in only one report; it has been shown that PX increases the mitotic activity of thyroid follicular cells[14]. The role of lighting conditions in the control of thyroid growth remains, so far, unclear[11,12,15]. The aim of the present study was to examine the influence of different lighting conditions on the mitotic activity of thyroid follicular cells in mice with an intact pineal.

Materials and methods. Eleven adult male mice of C57 bl strain (mean weight = 38 ± 5 g) were used in the study. Six mice were subjected to long photoperiod (LPP) (16 h light: 8 h darkness) and the remaining 5 mice were exposed to short photoperiod (SPP) (10 h light: 14 h darkness). The animals were maintained in lighting conditions specified above for 10 weeks. In order to evaluate the thyroid mitotic activity, the metaphase-arrest technique was employed. On the day of termination of the experiment the mice were injected with Colchicine (Sigma) at a dose of 1 µg/l g BW, i.p. Two hours later the mice were anesthetized with methoxyflurane (Metofane, Pittman-Moore) and bled by cardiac puncture. Thyroid glands were collected from all the animals and fixed in 10% formalin. After histological processing, paraffin sections (6 µm thick) were stained with hematoxylin and eosin. In the microscopic preparations the mitotic activity rates (number of metaphases per 1,000 scored follicular cells from the randomly selected sections of each thyroid lobe) were evaluated. Groups to be compared were tested for equality of variances using the F-test. A difference in mean mitotic activity rates (MMARs) between groups was estimated by means of the Student's t-test.

Results and discussion. The results are presented in figure 1. As shown, the MMAR of the thyroid follicular cells is significantly lower in mice which were maintained in SPP



Mean mitotic activity rates in the mice subjected to short photoperiod (SPP) and long photoperiod (LPP). Bars represent means ± SEM; p, level of significance.