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DIFFERENT REPLICATION PATTERN OF TERTIARY AND QUATERNARY STRUCTURE CHROMATIN IN REGENERATING RAT LIVER

Rüdiger Brust and Eberhard Harbers

Institut für Medizinische Molekularbiologie
der Medizinischen Hochschule Lübeck
Ratzeburger Allee 160, D-2400 Lübeck (G.F.R.)

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SUMMARY

In the regenerating rat liver chromatin of predominantly quaternary structure is preferentially replicated during the late S-phase (26 - 30 hrs after partial hepatectomy), while tertiary structure chromatin is mainly replicated in the early S-phase (16 - 20 hrs after partial hepatectomy).

INTRODUCTION

Chromatin has a highly preserved structural order when isolation has been done under conditions avoiding drastic changes of ionic strength during lysis of the cell nuclei. Small angle X-ray scattering studies indicated that such material contains quaternary structure chromatin which seems to be a solenoidal arrangement of nucleosomes with an outer diameter of about 32 - 34 nm (hydrated chromatin in solution), and in addition tertiary structure chromatin (i. e. nucleosomal chains). By gel-chromatography this chromatin can be fractionated. The first tubes then contain mainly quaternary structure chromatin with low content of tertiary structures; in the subsequent tubes the ratio of the contents of tertiary to quaternary structure increases (1). This paper is a report on kinetic studies with [^{3}H]-deoxythymidine in order to elucidate the replication be-

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haviour of quaternary and tertiary structure chromatin in the regenerating rat liver after partial hepatectomy.

MATERIALS AND METHODS

Preparation of chromatin

Rat liver cell nuclei were isolated by the method of POGO et al. (2). Soluble chromatin was prepared by a modification of the method described by REES et al. (3). Sedimented nuclei of about 14 g liver were washed 2 x in 0.25 M sucrose, then dispersed in 2.5 ml 0.25 M sucrose and mixed with 2.5 ml incubation buffer (0.04 M Tris, 0.08 M K_2SO_4, pH 7.4, adjusted by adding H_2SO_4). Incubation at 38°C was started with addition of 50 µl of 0.05 M $MgSO_4$ and stopped after 30 minutes by adding 130 µl 0.1 M EDTA and chilling on ice. Crude chromatin was separated from insoluble material by centrifugation (5000 - 6000 g for 5 min).

Fractionation of chromatin

Soluble chromatin was dialysed against chromatography-buffer (0.1 M KCl, 0.02 M Tris, 0.005 M citric acid, pH 7.4) and then applied to a 2.5 cm x 40 cm column packed with Pharmacia Sepharose 2B. Elution was done at 4°C with chromatography-buffer at a flow rate of about 4 ml/hr. Fractions of 50 drops (about 3 ml) were collected. Absorbance was measured at 260, 280 and 230 nm. By determination of the DNA with the diphenylamine reaction (4) it was proved that the value of A_{260} is indeed proportional to the amount of DNA in chromatin.

Identification of tertiary and quaternary structure chromatin

Identification and a semi-quantitative approximation of the relative contents of tertiary and quaternary structures in chromatin solutions were done by small angle X-ray scattering (SAXS). Experimental conditions of the SAXS-measurements and the evaluation of the data are described in detail in a foregoing paper (1). In the first tubes of gel-chromatography chromatin is mainly organized in quaternary structure, with increasing tube numbers the relative amount of tertiary structure chromatin is increasing.

Labeling of the DNA of rat liver chromatin after partial hepatectomy

For each single experiment 6 female Wistar-rats (100-130 g) were partially hepatectomized under light ether anaesthesia (5). Two hours before sacrificing 12.5 µCi [^{3}H]-deoxythymidine were injected intraperitoneally. In order to avoid any influence of the day-night rhythm on the regeneration process in all experiments partial hepatectomy was done at the same day-time. Chromatin preparation and fractionation by gel-chromatography was done as described above. Aliquots from the tubes were mixed with Packard Insta-gel or Baker Aqua Luma, respectively; radioactivity was measured in a Berthold Liquid Scintillation Counter. Within each single experiment quenching effects were proved to be constant for all samples to be measured.

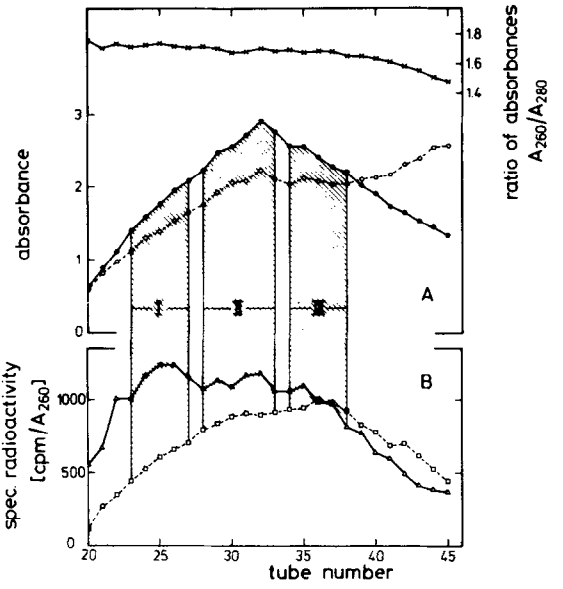

Fig. 1
Fractionation of rat liver chromatin by gel-chromatography.

A: The chromatin-peak (tube no. 20 to 38) is indicated by absorbance A_{260} (●—●) being higher than A_{230} (○-○-) or by the ratio of absorbances A_{260}/A_{280} (×—×) having a value of about 1.7. In tubes of higher numbers than 38 the relative amount of protein is increasing: A_{230} exceeds A_{260}, the ratio A_{260}/A_{280} is decreasing to values of about 1.5. According to the results of SAXS-studies (1) the chromatin-peak is divided into three fractions: pool I,II and III.

B: Specific radioactivity [cpm/A_{260}] is plotted against tube numbers; animals were sacrificed 20 hrs (○-○-) and 26 hrs (△—△) after partial hepatectomy. DNA of chromatin is labeled by incorporation of [^{3}H]-deoxythymidine. The specific radioactivity within pool I is clearly higher for later chromatin preparation.

RESULTS

The method applied for chromatin preparation from normal rat liver yields heterogeneous chromatin of high molecular weight with s-values of about 90. Purification and fractionation by gel-chromatography is demonstrated in Fig. 1A. Analytical determinations resulted in an averaged ratio histone:non-histone-protein:RNA:DNA = 1.1:0.5:0.03:1 for the material in tubes 20 to 38, in agreement with well known results on chromatin. In accordance, this chromatin-peak is indicated by the absorbances A_{260} being higher than A_{230}, and additionally by the ratio

A_{260}/A_{280} having a constant value of about 1.7. The subsequent tubes (> 38) contain decreasing amounts of DNA and increasing amounts of proteins. Early eluted material has high s-values of about 150, indicating this chromatin as being of a higher molecular order. Within the chromatin-peak the s-values decrease to nearly 60 with increasing tube numbers.

For structural studies by SAXS chromatin, concentration must be at least 1 mg/ml. Therefore, tube contents were pooled as indicated with I, II and III in Fig. 1A and concentrated with Millipore immersible ultrafiltration units. The results of SAXS-investigations are interpreted as chromatin in pool I being mainly organized in quaternary structure, the content of tertiary structure chromatin being fairly low; with increasing tube numbers the amount of tertiary structure is increasing (1).

In Fig. 1B specific radioactivity of fractionated chromatin prepared from regenerating rat liver is plotted against tube numbers 20 hrs and 26 hrs after partial hepatectomy. 20 hrs after partial hepatectomy, less specific radioactivity appears in the first tubes of the chromatin-peak, but with increasing tube numbers increasing values are obtained; thus, specific radioactivity is higher in pool III than in pool I. 26 hrs after partial hepatectomy, the results are quite different: specific radioactivity in the first tubes is higher, with increasing tube numbers it remains nearly constant, while it is slightly lower in the tubes of pool III. These differences can be demonstrated much clearer by the ratio of averaged specific radioactivity of pool III to that of pool I when plotted against time after partial hepatectomy (Fig. 2). The ratio is high at 16 hrs, indicating higher specific radioactivity in pool III (containing chromatin with higher amount of tertiary

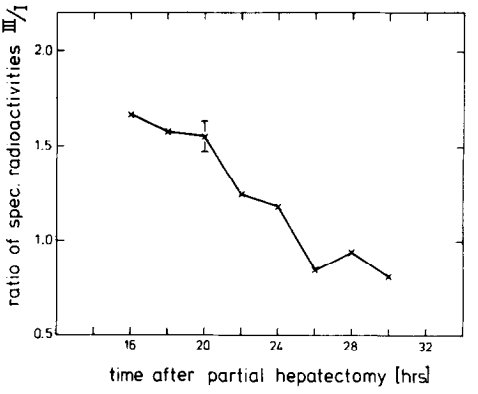

Fig. 2

Ratio of specific radioactivities of pool III to that of pool I $[(cpm/A_{260})_{III}/(cpm/A_{260})_{I}]$, plotted against time [hours after partial hepatectomy]. The bar indicates deviation of the experimental points derived from two experiments done under identical conditions.

structures). In contrast, material in pool I containing mainly quaternary structure chromatin becomes intensively labeled in the later phase of regeneration (26 - 30 hrs after partial hepatectomy).

DISCUSSION

Absolute values of DNA-labeling in chromatin are very low at 16 hrs after partial hepatectomy, they increase corresponding to the maximum of histone and DNA-synthesis during the period of 20 - 26 hrs (6,7). Although DNA-synthesis is not perfectly synchronized at this time, there is such an accumulation of cells in S-phase that certain correlation with the cell cycle, including early and late replication of DNA, can be detected. The easiest way for an approximate comparison of the rates of DNA-synthesis in quaternary (pool I) and tertiary (pool III) chromatin structures is to form the ratio of DNA specific radioactivity of these two fractions. If this is done for each single experiment, variations can be eliminated which may appear as a consequence of slight differences in the dosage of the la-

beled precursor or its resorption after intraperitoneal injection.

From the results demonstrated in Fig. 2 it is evident that in the early S-phase fraction pool III has a much higher specific radioactivity in comparison to fraction pool I. During the subsequent period of the S-phase this ratio is decreasing, and finally it is almost reversed.

It should be noted that statements based on these results cannot be completely unequivocal. Gel-chromatography does not permit a perfect separation of quaternary from tertiary structure chromatin; in pool I quaternary structures are accumulated with fairly low content of tertiary structures, in pool III the amount of tertiary structures is higher than in pool I. In spite of this restriction the described results indicate that in the early S-phase preferential replication of chromatin organized as tertiary structure takes place, while during later S-phase replication of quaternary chromatin apparently is dominating. This pattern of replication corresponds quite well to that of euchromatin and heterochromatin as previously observed in mammalian tissues (8,9,10). As yet, one cannot decide how far these two kinds of chromatin are really identical with material now characterized as tertiary and quaternary structure chromatin.
An additional suggestion for such an analogy is apparently the pattern of transcription. Preliminary results of RNA-DNA-hybridization experiments (11) indicate that in rat liver cells nuclear transcription mainly takes place at tertiary structure chromatin which then has to be considered as active chromatin.

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