DIFFERENCES IN ULTRASTRUCTURE OF SUBUNITS OF COMPACT AND DIFFUSE CHROMATIN

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The ultrastructure of subunits of compact and diffuse chromatin of isolated Wistar rat hepatocyte nuclei was studied. The complex structure of the chromatin subunits, which consist of a central nucleosome surrounded by a capsule, was confirmed. The capsule was shown to exist in either a compact or a diffuse state, and this determines the compact or diffuse state of the chromatin. It is suggested that the capsule of the nucleosome in its diffuse form is the site of transcription.

KEY WORDS: *compact and diffuse chromatin*; *nucleosomes*; *ultrastructure*.

Chromatin is known to consist of discrete particles or nucleosomes [9]. Although the morphological and biochemical characteristics of active (as regards transcription) and inactive chromatin differ [3, 6], no difference has been found between the nucleosomes of active and inactive chromatin [4, 5]. The absence of such differences, it can tentatively be suggested, is due to the unification of the chromatin subunits in the course of their isolation, for the investigations cited above were carried out with isolated nucleosomes and the methods of their isolation involve procedures influencing the nucleic acid and protein components of the chromatin.

It is shown in this paper that the ultrastructure of the subunits of diffuse, i.e., active, chromatin when studied in situ in the nuclei differs from that of the subunits of compact (inactive) chromatin.

EXPERIMENTAL METHOD

Hepatocyte nuclei from Wistar rats were isolated by the sucrose method [1]. The nuclei were fixed in a 2.5% solution of glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, at room temperature for 1 h and then postfixed in a 2% solution of osmium tetroxide in the same buffer with the addition of 0.2 M sucrose at room temperature for 2 h, dehydrated in alcohols, and embedded in Epon. Ultrathin sections, stained with uranyl acetate and lead citrate, were studied in the EMV-100L electron microscope with an accelerating voltage of 75 kV.

EXPERIMENTAL RESULTS

Division of the chromatin into compact and diffuse still remained in the isolated nuclei (Fig. 1). Both consisted of discrete subunits, differing in their ultrastructure. The subunits of compact chromatin were uniform. They had the appearance of globules measuring 20-25 nm, with the high electron density (Fig. 2a). Each such chromatin globule was surrounded by a pale zone with a filamentous structure, making the outlines of the particle indistinct. The thickness of the pale zone varied from 5 to 7.5 nm. If this zone was regarded as a component of the globule, the dimensions of the latter were 30-40 nm, corresponding to the largest size of isolated chromatin subunits given in the literature (37 ± 7 nm [8]). In places short chains of globules, either tightly packed together or connected by a filament up to 5 nm long, could be seen: these were segments of chromatin fibrils located in the plane of the section.

The subunits of diffuse chromatin are highly variable. Their dense central part is smaller and their diffuse peripheral zone wider than in compact chromatin (Fig. 2b). In the center of the dense part of most globules of diffuse chromatin there is a round clear space

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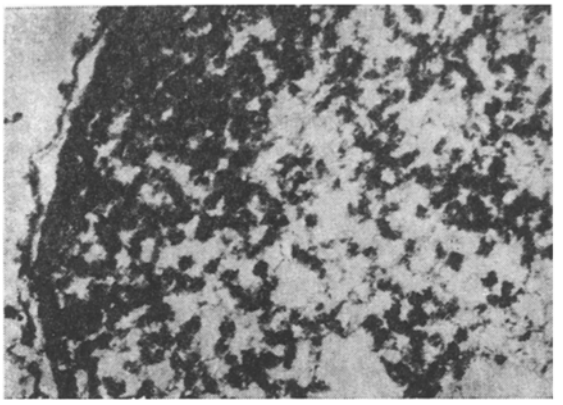

Fig. 1

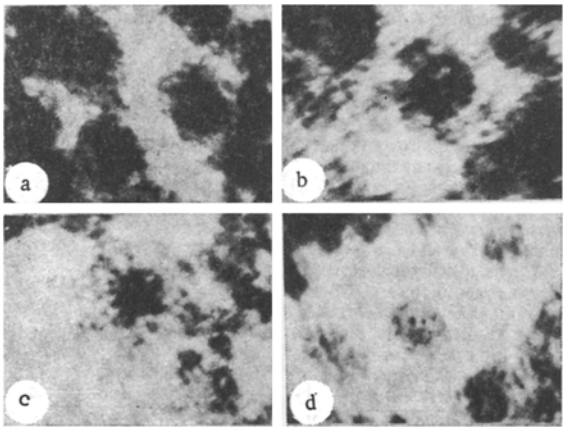

Fig. 2

Fig. 1. Ultrastructure of isolated rat hepatocyte nucleus, 80,000×.

Fig. 2. Chromatin subunits of isolated nucleus: a) subunits of compact chromatin; b-d) subunits of diffuse chromatin, 300,000×.

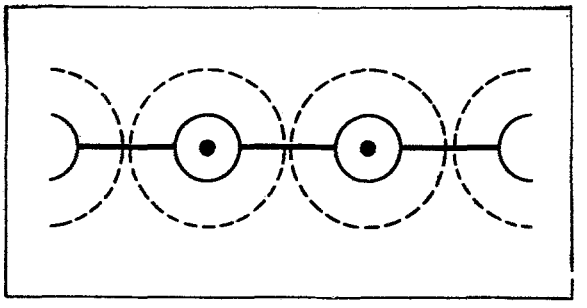

Fig. 3. Scheme of structure and mutual arrangement of chromatin subunits (β particles). Each subunit contains one nucleosome (continuous ring), surrounded by a capsule. The nucleosomes are connected into a chain by a DNA molecule.

10-12.5 nm in diameter, with a dark granule measuring about 2.5 nm in the center (Fig. 2c). It was difficult to determine the overall size of these globules because of the indistinctness of their external outline, on all sides twisted filaments 2-2.5 nm thick spread out from the diffuse part. The subunits of diffuse chromatin, which were loosely packed, had the appearance of a thin-walled ring 10-12.5 nm in diameter, with a central granule surrounded by a wide diffuse zone (Fig. 2d). These rings corresponded in their morphology to the isolated γ particles (nucleosomes) [7].

On the basis of the results of a study of the ultrastructure of chromatin after histone blockage by heparin, the writers [2] postulated the existence of two components of chromatin subunits — the nucleosome and the capsule surrounding it. The results of the present investigation confirm the existence of these components. In diffuse chromatin, in which the chromatin globules are in a physiologically loosely packed state, both the nucleosome (the central sphere) and its capsule (the diffuse peripheral zone) can be seen. Consequently, the chromatin globule 20-25 nm in diameter is a combined formation, to describe which the term "β particle" is suggested, whereas the chromatin fibril is a band of β particles, connected with each other by a DNA molecule, packed in a single row (Fig. 3).

The capsule of the nucleosome may be in either the compact or the diffuse state, and this determines the compact or diffuse state of the chromatin. The suggestion that DNA participating in transcription is concentrated in the capsule is a valid one. The nucleosome evidently does not participate in functional changes in chromatin structure, and this explains observations indicating that the nucleosomes of compact and diffuse chromatin are identical.

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