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Ultrastructural distribution of DNA and RNA within the nucleolus of human Sertoli cells as seen by molecular immunocytochemistry

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SUMMARY

The precise distribution of DNA and RNA within the human Sertoli cell nucleolus has been investigated, at the ultrastructural level, by cytochemical and molecular immunocytochemical techniques. In Sertoli cells, the nucleolar components show a typical spatial distribution. The fibrillar centres are not surrounded by a layer of dense fibrillar component, but come in contact only with strands of dense fibrillar component. These fibrillar parts of strands are the extensions of granular strands connected to a large granular mass. These strands delimit numerous nucleolar interstices in which chromatin fibres are clearly obvious. Using the in situ terminal deoxynucleotidyl transferase/immunogold procedure for detecting DNA, we find evident label exclusively over the chromatin fibres enclosed in the nucleolar interstices and over the fibrillar centres, and no significant label over the dense fibrillar component and granular component of the nucleolus. Furthermore, using the polyadenylate nucleotidyl transferase/immunogold procedure for detecting RNA, we show that label is deposited not only over the granular component and dense fibrillar component, as expected, but also quite obviously over the fibrillar centres. No label is seen over the interstices containing chromatin.

Key words: nucleolus, Sertoli cells, immunocytochemistry

INTRODUCTION

On the basis of autoradiographic studies, it used to be accepted that rDNA transcription takes place in the dense fibrillar component of the nucleolus (reviewed by Goessens, 1984; Fakan, 1986). Over the past few years, however, data based on immunolocating procedures at the electron microscope level have strongly suggested that rDNA transcription occurs in the fibrillar centres (Scheer and Benavente, 1990; Thiry et al., 1991). A variety of methods, applied to various cell-type nucleoli, have detected DNA exclusively in the fibrillar centres, preferentially in their peripheral regions, but not in the dense fibrillar component (Scheer et al., 1987; Thiry, 1988, 1992; Thiry et al., 1988, 1991, 1992).

Recent in situ hybridization studies have, however, yielded apparently contradictory results concerning the location of rRNA genes in the nucleolar fibrillar components. In Ehrlich tumour cells (Thiry and Thiry-Blaise, 1989, 1991), HeLa cells, and mouse 3T3 cells (Puvion-Dutilleul et al., 1991), rDNA was detected only in the fibrillar centres and not in the dense fibrillar component, while the reverse was observed for human Sertoli cells (Wachtler et al., 1992) and human lymphocytes (Wachtler et al., 1990).

Although the functional organization of the nucleoli may be different in different cells or species, we have put forward the hypothesis that the divergent conclusions might arise from difficulties in distinguishing the various nucleolar components under in situ hybridization conditions (Thiry and Goessens, 1992). In this respect, we have recently shown that the rDNA signal obtained after in situ hybridization on human lymphocytes should be attributed to the condensed chromatin crossing through the dense fibrillar component rather than to the dense fibrillar component itself (Vandelaer et al., 1992).

To shed light on the nucleolus of human Sertoli cells, we have used the in situ terminal deoxynucleotidyl transferase/immunogold (TdT/immunogold) technique to investigate, in great detail, the precise distribution of DNA within the nucleolus. This method was applied to acetylated material, conditions that provide an excellent distinction between the various nucleolar components and make it possible to visualize condensed chromatin with high contrast. To obtain additional information concerning the morphofunctional organization of the nucleolus, the distribution of RNA was further investigated by means of the in situ polyadenylate nucleotidyl transferase/immunogold (PnT/immunogold) technique.

The results reveal DNA in the chromatin enclosed in the nucleolar interstices, especially those in contact with the fibrillar centres, as well as in the fibrillar centres, but not in the dense fibrillar component of the human Sertoli cell nucleolus. Moreover, our results indicate that the fibrillar centre is the only site where DNA and RNA are visualized together.

MATERIALS AND METHODS

Testicular material from human adults was obtained by courtesy of Dr P. Delrée (University of Liège, Liège, Belgium).

Electron microscopy

Small fragments of testicular material were fixed for 60 min at 4°C in 1.6% glutaraldehyde in 0.1 M Sorensen's buffer (pH 7.4), acetylated as previously described (Wassef et al., 1979), and embedded in Epon. Ultrathin sections were mounted on nickel grids and stained with uranyl acetate and lead citrate before examination in a Jeol CX 100 electron microscope at 60 kV.

Detection of DNA and RNA

To pinpoint the precise location of DNA and RNA, respectively, the sensitive TdT/immunogold technique (Thiry, 1992a,b) and the sensitive PnT/immunogold procedure (Thiry, 1992c) and their controls were used as previously described.

Quantitative evaluation

To evaluate the labelling density, the area of each compartment studied was first estimated morphometrically by the point-counting method (Weibel, 1969). After evaluating the areas (S_a) occupied by the various compartments, we counted the number of gold particles (N_i) over each compartment and calculated the labelling density ($N_s = N_i/S_a$). In the present study, to evaluate the DNA and RNA labeling densities, 21 and 7 random micrographs were analyzed, respectively and 5,645 and 2,986 gold particles counted, respectively. We must bear in mind that these numerical data do not reflect the exact amounts of nucleic acid molecules in the compartments studied; thus, only relative intensities between compartments can be considered. This quantitative evaluation allows only a demonstration of the specificity of the labelling.

RESULTS

Morphology

On electron micrographs, the human Sertoli cell nucleus is easily recognized by the following criteria. The chromatin appears uniform, distributed throughout the nucleus, and essentially constituted of fibres approximately 25 nm in diameter. The nuclear envelope shows many deep invaginations. A single nucleolus stands out very clearly and shows a typical arrangement.

Three distinct parts form the nucleolus (Fig. 1A,B): one or sometimes two large fibrillar centres, a granular mass, and strands containing dense fibrillar component and granular component. These strands connect the fibrillar centres with the granular mass. Only those parts of the strands that contain dense fibrillar component come in contact with the fibrillar centres. Apart from these connections, the fibrillar centres appear separated from the other nucleolar components by nucleolar interstices. Numerous nucleolar interstices are also found between the strands themselves, and between the strands and the granular mass. Inside these spaces, chromatin fibres or a few rare, small clumps of condensed chromatin are consistently observed. This chromatin can be visualized in close contact with the fibrillar centres.

Location of DNA within the nucleolus

When the in situ TdT/immunogold technique is applied to acetylated cells, gold particles are found scattered throughout the nucleoplasm (Fig. 2A). Only the interchromatin granules appear free of gold particles.

Inside the nucleoli (Fig. 2A,B), label is preferentially seen over the nucleolar interstices, notably those bordering on the fibrillar centres. Evident label is also consistently present over the fibrillar centres, centrally as much as peripherally. In contrast, although gold particles are frequently observed at junctions between the strands and the intersitices, no label is visualized over the fibrillogranular strands themselves. The granular masses are also devoid of label.

Numerical data on gold particle distribution over the various nucleolar components are summarized in Table 1. They confirm the subjective observations and further establish the high specificity of the labelling. In fact, the labelling density over the nucleolar interstices approaches the value measured over the nucleoplasm. Although much lower than that obtained over the nucleolar interstices, significant label is also found over the fibrillar centres. Over the dense fibrillar component and the granular component, on the other hand, the labelling density is insignificant.

Location of RNA within the nucleolus

To pinpoint the location of RNA within the nucleolus, the in situ PnT/immunogold procedure was applied to acetylated cells. Under these experimental conditions, both cytoplasmic and nuclear compartments of interphase cells are labelled. In the nuclei, label is particularly obvious over the nucleoli (Fig. 3). Inside all the nucleoli, evident labelling is present over the dense fibrillar component and the granular component. In the fibrillogranular strands, gold particles are consistently visualized both centrally and peripherally. Label is also detected over all the fibrillar centres. Very faint labelling is seen over some nucleolar interstices. When a nucleolar interstice contains a small clump of condensed chromatin, the latter is never labelled.

Numerical data on gold particle distribution over the various nucleolar components are summarized in Table 2. They confirm the subjective observations and further establish the high specificity of the labelling as illustrated by the absence of gold particles over the rare clumps of condensed chromatin. In fact, the labelling density over the fibrillar centers, although higher than that obtained over the granular component, has a value near to the labelling density over the dense fibrillar component of the nucleolus. Over the nucleolar interstices, on the other hand, the labelling density is clearly lower. The labelling density over the nucleoplasm has a value similar to that measured over the granular component.

DISCUSSION

The present study shows that DNA is present in the nucleolar interstices and the fibrillar centres of human Sertoli cell nucleoli. As for the dense fibrillar component, it appears to be completely devoid of DNA.

These results appear to contradict the recent data obtained on the same cell type by in situ hybridization (Wachtler et al., 1992). In fact, the in situ hybridization signal was attributed to the dense fibrillar component

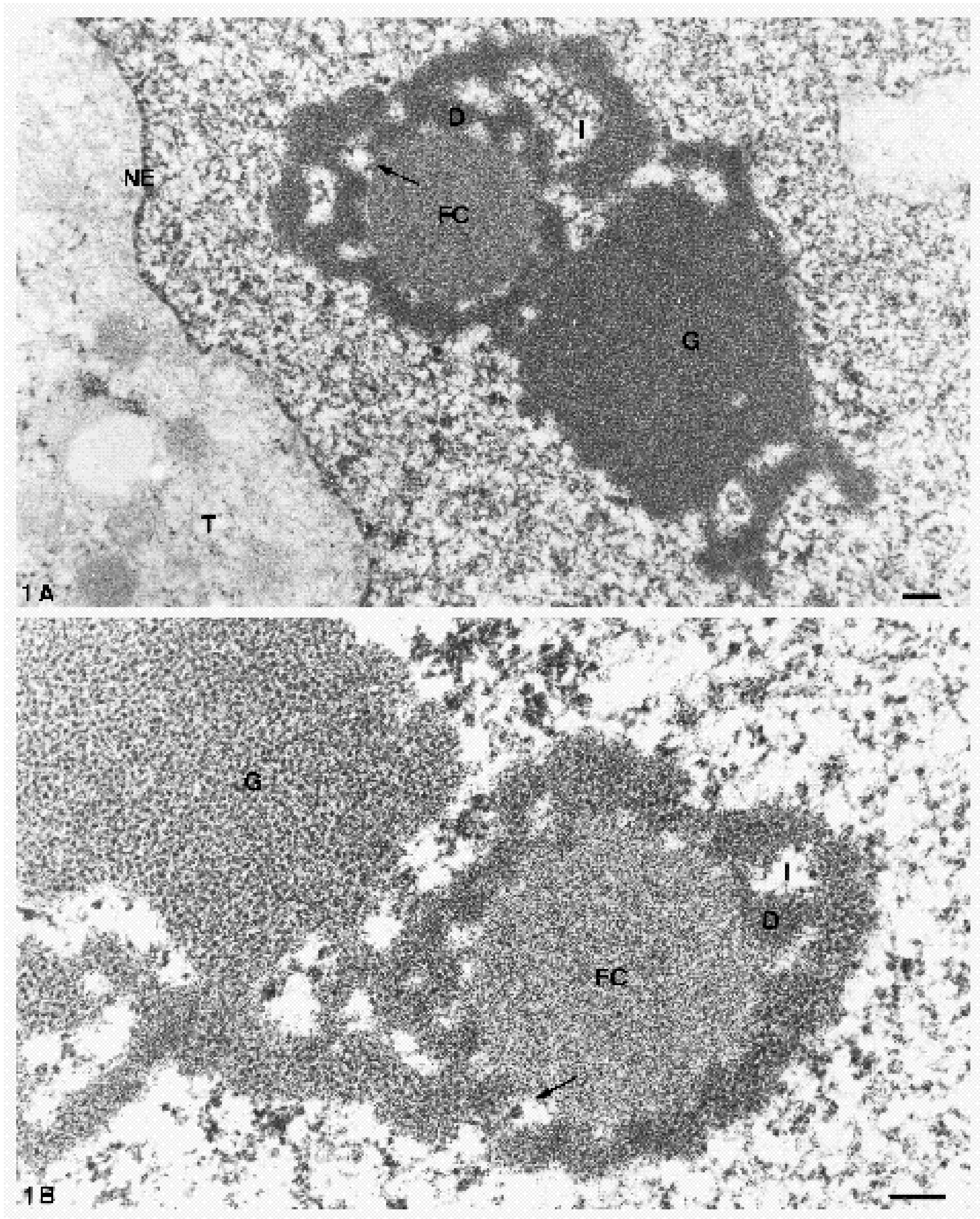

Fig. 1. Nucleoli of human Sertoli cells after acetylation. (A) General view. (B) detail of a fibrillar centre (FC) and of surrounding strands comprising dense fibrillar component (D) and granular component (G). These strands connect the fibrillar centre with a large mass formed exclusively by granular component. Note that the fibrillar centre is not completely surrounded by dense fibrillar component but in contact with it only at a few sites. Chromatin is clearly seen in nucleolar interstices (I), in particular those in contact with the fibrillar centres (arrows). NE, nuclear envelope; T, cytoplasm. Bars, 0.2 μm.

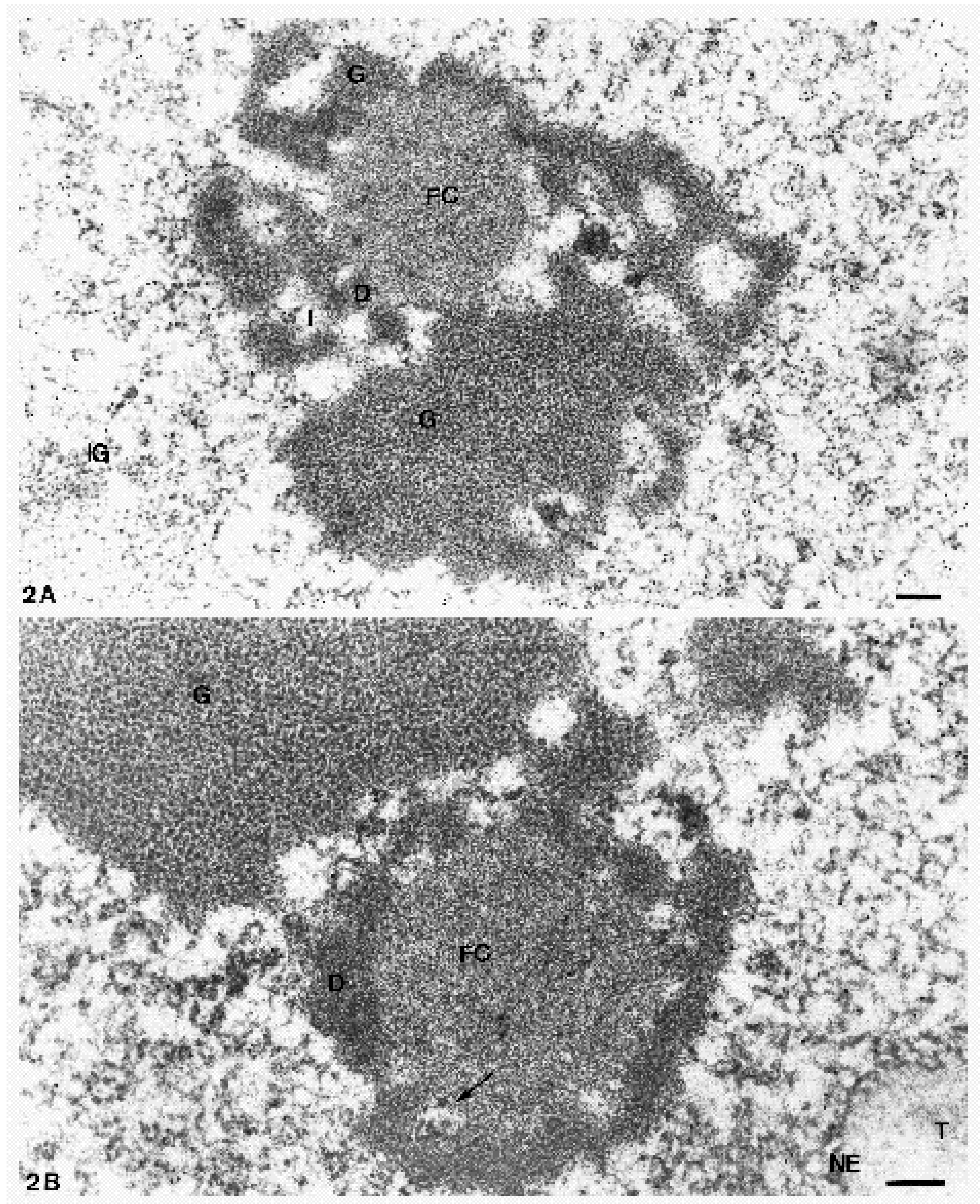

Fig. 2. Nucleoli of human Sertoli cells after acetylation combined with the in situ TdT/immunogold technique for detecting DNA in situ. (A) General view. (B) Detail of a fibrillar centre (FC) and its surrounding regions. Gold particles are clearly seen over the chromatin enclosed in the numerous nucleolar interstices (I), in particular in those in contact with the fibrillar centres (arrow). Label is also found over the fibrillar centres. By contrast, the dense fibrillar component (D) and the granular component (G) are gold-free. IG, interchromatin granules; NE, nuclear envelope; T, cytoplasm. Bars, 0.2 µm.

Table 1. Labelling densities (gold particles per µm²) after applying the TdT/immunogold procedure on human Sertoli cell sections

	Mean values ± s.e.m.	'Corrected' values*
Nucleolus		
Fibrillar centres	8.352±3.395	6.381
Dense fibrillar component	1.931±2.115	0
Granular component	1.906±1.137	0
Interstices	48.163±28.218	46.192
Nucleoplasm	59.149±32.901	57.178
Cytoplasm†	1.971±1.492	0

*The 'corrected' values were obtained by subtracting the background (density of labelling on the cytoplasm).
†Excepting the mitochondria.

because these authors considered that the strands were entirely composed of dense fibrillar component in the nucleoli of human Sertoli cells. However, as shown here and in previous studies (Devictor et al., 1984, 1987) the strands are not exclusively formed by dense fibrillar component but comprise alternating segments of dense fibrillar component and granular component. On the other hand, these strands take on a reticular appearance due to the presence of numerous interstices that the present work shows to contain chromatin. Although Wachtler et al. (1992) frequently observed label at the periphery of these strands and, thereby, as shown here, at the periphery of the nucleolar interstices, they took no account of the nucleolar interstices, perhaps because they were hard to identify under in situ hybridization conditions.

Accordingly we think that the rDNA signal obtained after in situ hybridization on human Sertoli cell nucleoli should be attributed to the chromatin enclosed in the nucleolar interstices rather than to the dense fibrillar component itself. In this regard, it is pertinent to note that, apart from a few cytochemical studies suggesting the presence of DNA inside the dense fibrillar component, DNA has never been clearly demonstrated there (discussed by Thiry and

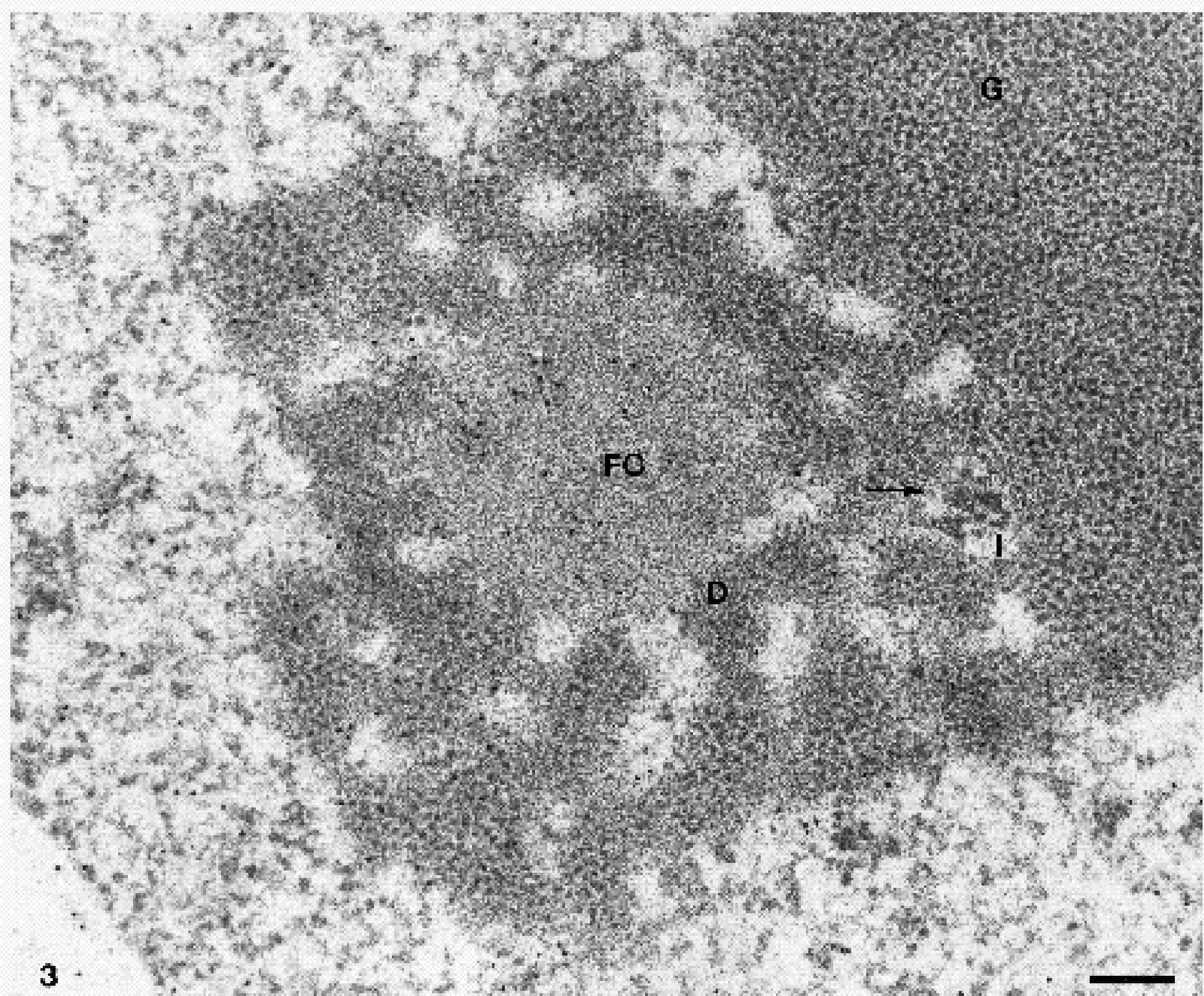

Fig. 3. Nucleolus of a human Sertoli cell after acetylation combined with the in situ PnT/immunogold technique for detecting RNA in situ. Besides the labelling of the dense fibrillar component (D) and granular component (G), gold particles are clearly seen over the fibrillar centre (FC). On the contrary, the nucleolar interstices (I) containing chromatin appear completely devoid of label (arrow). Bar, 0.2 µm.

Table 2. Labelling densities (gold particles per μm²) after applying the PnT/immunogold procedure on human Sertoli cell sections

	Mean values±s.e.m.
Nucleolus	
Fibrillar centres	40.14±15.97
Dense fibrillar component	40.66±9.21
Granular component	25.40±6.43
Interstices	6.33±2.85
Condensed chromatin	0
Nucleoplasm*	22.41±4.79

*Nucleoplasm comprises all nuclear areas outside the nucleolus and the few rare clumps of condensed chromatin.

Goessens, 1992). Moreover, the presence of rRNA genes in the intranucleolar chromatin enclosed in interstices is in complete agreement with previous data obtained on a variety of cell types in both biochemical (Bachellerie et al., 1977) and in situ hybridization studies (Jacob et al., 1974; Thiry and Thiry-Blaise, 1989, 1991; Puvion-Dutilleul et al., 1991, 1992; Stahl et al., 1991). In Ehrlich tumour cell nucleoli, we have even previously pointed out the presence of rDNA in the condensed chromatin interrupting the dense fibrillar component and in contact with the fibrillar centres (Thiry and Thiry-Blaise, 1989, 1991).

These results extend our previous observations obtained on other cell types, concerning the distribution of DNA within the nucleolus (Thiry, 1992a); they also emphasize the fact that it is important to know the fine distribution of DNA within the nucleolus before investigating the distribution of more specific DNA segments associated with the nucleolus.

The present study also shows that RNA is present in the fibrillar centres of the human Sertoli cell nucleolus but not in nucleolar interstices containing chromatin. The presence of RNA in the fibrillar centres is in complete agreement with recent data obtained on a few other cell types by the PnT/immunogold technique, and by postembedding immunogold labelling procedures using two different anti-RNA antibodies (Thiry, 1992b). In Ehrlich tumour cell nucleoli, moreover, we have recently demonstrated the presence of appreciable amounts of rRNA in the fibrillar centres by electron microscope in situ hybridization (Thiry, 1992c).

The fibrillar centres of the human Sertoli cell nucleolus appear, as in other cell types, to be the only nucleolar component in which significant amounts of both nucleic acids are visualized together. These results are in line with the view that rRNA synthesis takes place in the fibrillar centres of mammalian cell nucleoli and indicate further that the dense fibrillar component cannot be considered as formed by superposition of active rRNA genes and their transcripts, but should rather be seen as a component where primary transcripts are rapidly accumulated after their release from the rDNA template (Scheer and Benavente, 1990; Thiry et al., 1991).

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