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Location of DNA within the nucleolus of rat oocytes during the early stages of follicular growth

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ABSTRACT We have investigated the DNA distribution within the rat oocyte nucleolus during the early stages of follicular growth by means of the *in situ* terminal deoxynucleotidyl transferase method. In the fibrillogranular nucleolus, label is visualized on small clumps of peri- and intranucleolar chromatin. Such labeled clumps are frequently observed inside the interstices surrounding the fibrillar centers. Label is also consistently found in the fibrillar centers whereas the dense fibrillar component and the granular component are devoid of gold particles. These results contradict earlier data but conform with other recent immunocytochemical observations, obtained in nucleoli of a variety of somatic cell types, concerning the correlation between structure and function in the nucleolus.

KEY WORDS: *nucleolus, DNA, oocyte*

Introduction

Because the nucleolus is the morphological expression of the activity of rRNA genes, it constitutes a very useful model for studying the relationships between structure and function. A generally accepted view is that ribosome biogenesis begins within the confines of the fibrillar components and continues in the granular component of the nucleolus (Goessens, 1984; Derenzini *et al.*, 1990; Scheer and Benavente, 1990; Thiry *et al.*, 1991). Much controversy, however, continues to focus on the precise location of transcriptionally active rRNA genes within the nucleolar fibrillar components (fibrillar centers and/or dense fibrillar component).

On the basis of autoradiographic studies of cells pulse-labeled with tritiated uridine, it was suggested that transcription of rRNA genes occurs in the dense fibrillar component of the nucleolus (for reviews, see: Goessens, 1984; Fakan, 1986). However, the use of a variety of immunocytochemical approaches for *in situ* DNA detection made it possible only to reveal DNA in the fibrillar centers and not in the dense fibrillar component (Scheer *et al.*, 1987; Thiry, 1988, 1991, 1992b; Thiry *et al.*, 1988; Thiry and Goessens, 1991). These techniques were nevertheless exclusively applied on nucleoli of various somatic cells.

In the present paper, we have extended this analysis to the nucleolus of germinal cells during the early stages of the follicular growth in rats. In mammals, during this period where the oocytes are arrested in the dictyate stage of the first meiotic prophase, a stage where the chromatin forms a diffuse network, an intense rRNA synthesis takes place in the oocytes (Moore *et al.*, 1974; Crozet *et al.*, 1981, 1986; Motlik *et al.*, 1984). To investigate the DNA distribution within reticulated nucleoli of rat oocyte, we have used the *in situ* terminal deoxynucleotidyl transferase (TdT) method, a very sensitive, high-resolution technique used to pinpoint the precise location of DNA under conditions favoring the clear distinction of the various nucleolar components (Thiry, 1992a).

Results

Morphology

As shown by Palombi and Stefanini (1974), Takeuchi (1984) and Antoine *et al.* (1987), from the primordial to the primary follicle stages, the rat oocyte nucleolus exhibits a fibrillogranular structure which is organized in a network separated by interstitial spaces. Small fibrillar centers are also clearly recognized. The latter are never completely surrounding by dense fibrillar component but present large contacts with interstices. Few condensed chromatin blocks appear to be associated with the nucleolus. Moreover, the chromosomes occur in the form of numerous small clumps of condensed material which are scattered in all the extranucleolar areas of the nucleus.

DNA detection

When the TdT method is applied on ultrathin sections of rat oocytes during the early stages of folliculogenesis, only a low nucleolar labeling is observed. Gold particles are essentially found on small clumps of condensed material which are located at the periphery of the nucleolus and inside the interstices, especially those surrounding the fibrillar centers. Label is also consistently present over the fibrillar centers. Inside the latter, gold particles are

Abbreviations used in this paper: TdT, terminal deoxynucleotidyl transferase.

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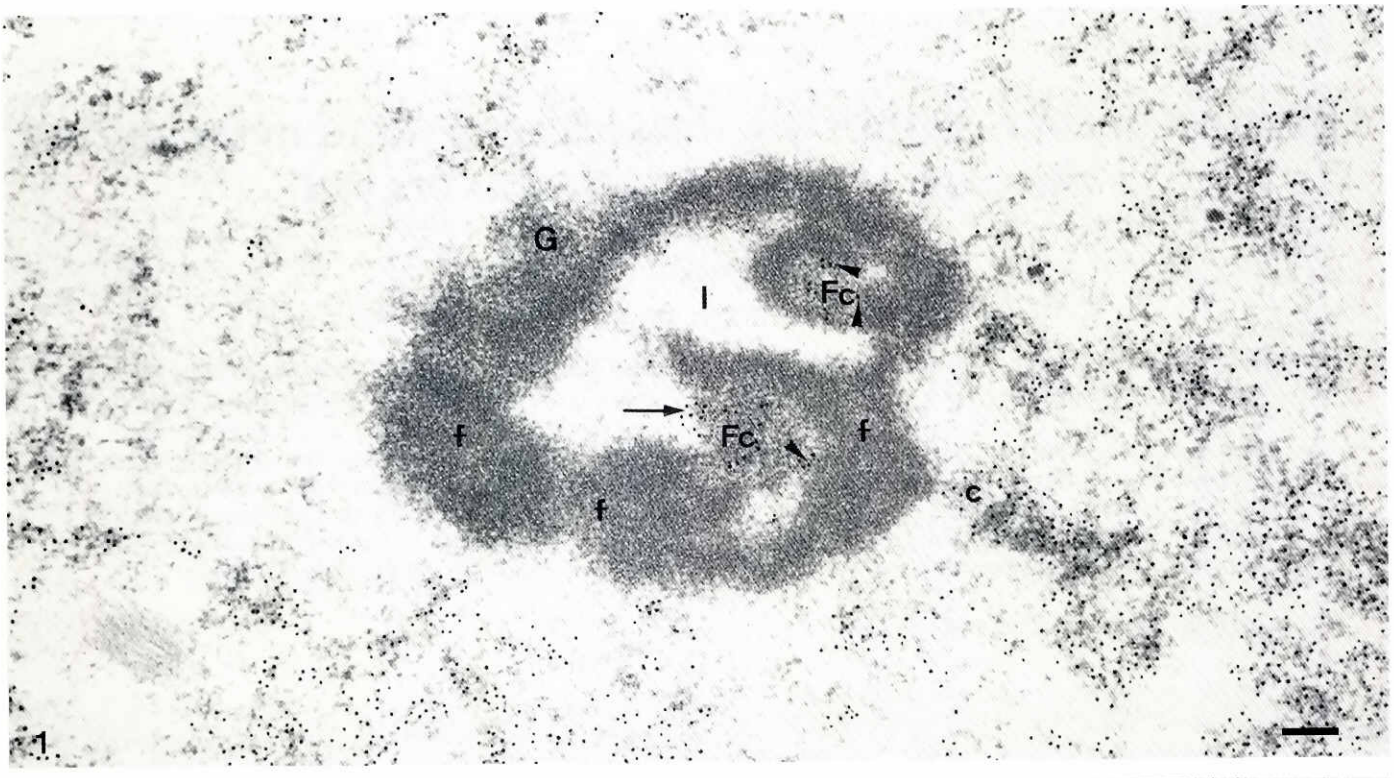
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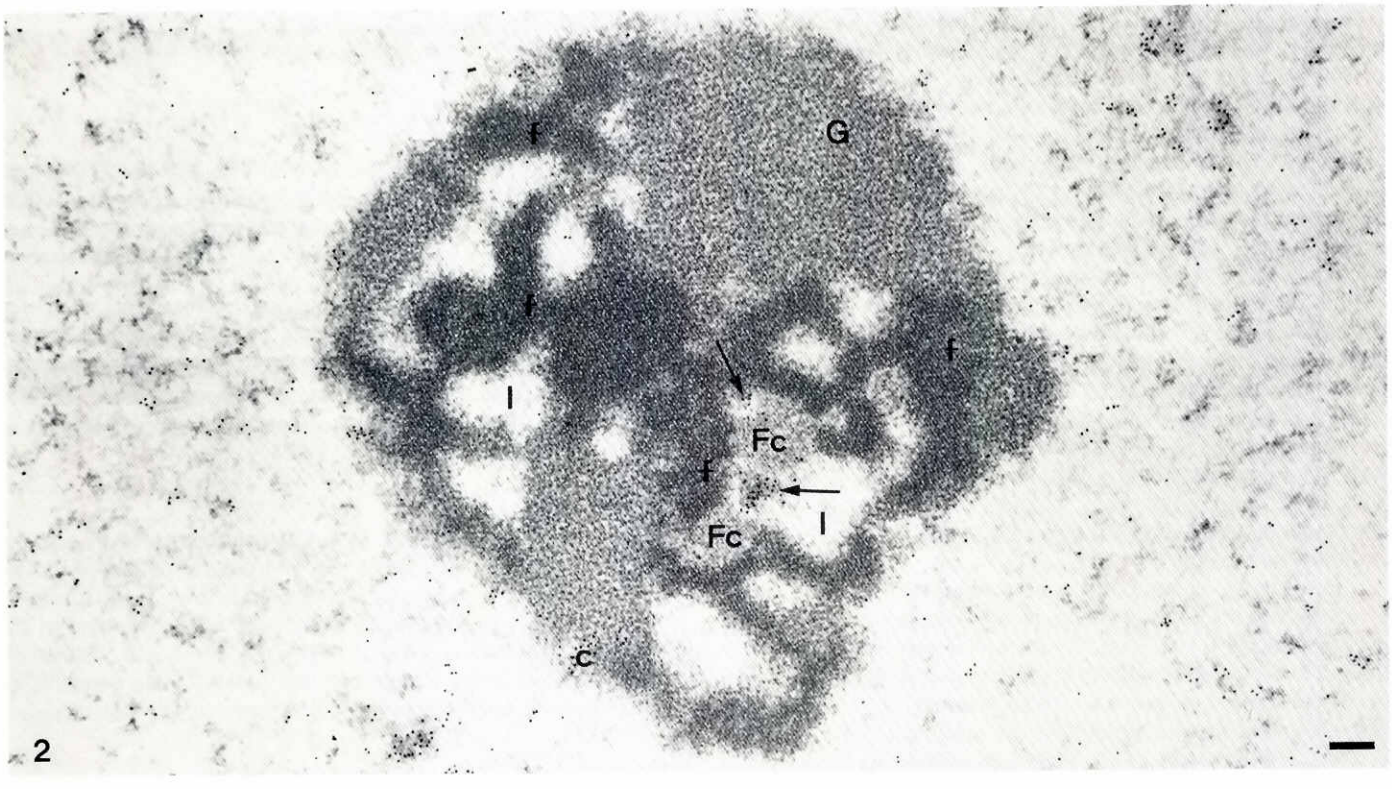
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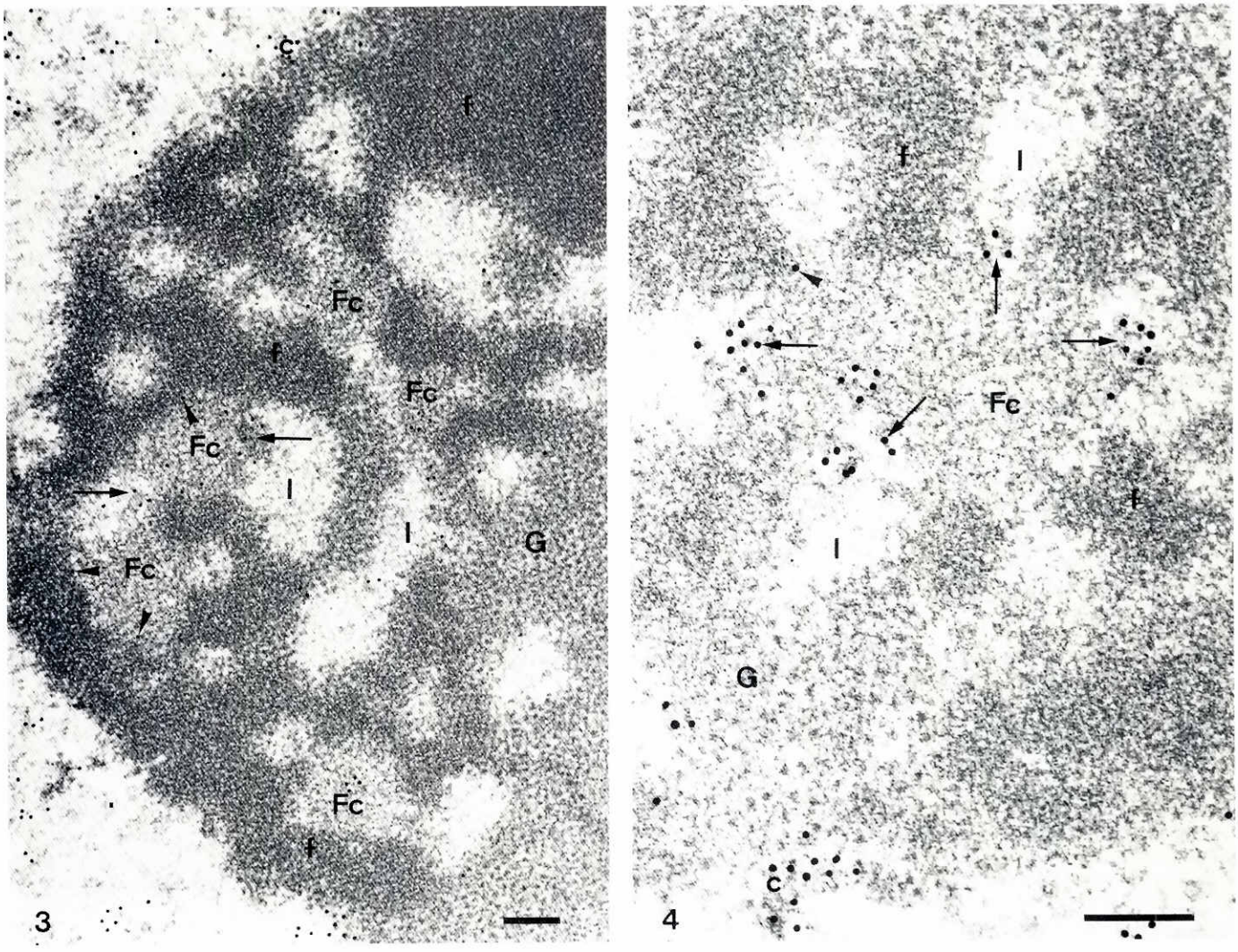

Figs. 1-4. Immunodetection of bromodeoxyribonucleotide triphosphates added by terminal transferase on ultrathin sections of rat oocyte nucleoli in a primordial (Fig. 1) or primary follicles (Figs 2-4). *Label is present on small clumps of condensed chromatin (c) either surrounding the nucleoli or enclosed in nucleolar interstices (l). Frequently, gold particle aggregates are visualized over nucleolar interstices in which small clumps of chromatin (arrows) approach the fibrillar centers (Fc). Label is also found in the fibrillar centers. Inside the latter, gold particles are seen at the limit (arrowheads) between the fibrillar centers and the dense fibrillar component (f). By contrast, no label is detected over the granular component (G) nor over the dense fibrillar component (f) of the nucleolus. Bars 0.2 μm.*

frequently found at the limit between the fibrillar centers and the dense fibrillar component. In contrast, the dense fibrillar masses themselves are unlabeled. The granular component is also gold-free.

Discussion

Using the *in situ* TdT method at the electron microscope level, we show that, inside the rat oocyte nucleolus during the early stages of follicular growth, DNA is preferentially located in the fibrillar centers and in the interstices surrounding them, whereas the well-developed dense fibrillar component appears completely devoid of label.

These observations are in total agreement with the DNA distribution obtained in the nucleolus from a variety of somatic cell types by means of the same approach (Thiry, 1992b) and other immunocytochemical techniques for *in situ* DNA detection (Scheer *et al.*, 1987; Thiry, 1988, 1991; Thiry *et al.*, 1988).

These results are not consistent with the classical concept, based on earlier autoradiographic studies, suggesting that the dense fibrillar component is the site of rRNA gene transcription (for reviews, see: Goessens, 1984; Fakan, 1986).

On the other hand, these results support the view that transcription occurs in the fibrillar centers while the dense fibrillar component could contain sites where primary transcripts are rapidly accumulated after their release from the DNA template (for reviews, see: Derenzini *et al.*, 1990; Scheer and Benavente, 1990; Thiry *et al.*, 1991).

Immunocytochemical research now in progress should supply new information on the evolution of the oocyte nucleolus during the next stages of follicular growth which are characterized by a complete change in nucleolar ultrastructure and by a drastic decrease in rRNA synthesis (Moore *et al.*, 1974; Crozet *et al.*, 1981, 1986).

Materials and Methods

Material

The oocytes originated from 28-day-old Wistar rats. After a Folligon («Intervet») stimulation (20 units FSH injected subcutaneously), ovaries were collected 42 h after injection. Some of them were clearly cut in small fragments in which oocytes at various stages of follicular growth were present.

Preparation for electron microscopy

Small fragments of ovaries were fixed for 30 min at 4°C with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). After washing in sodium cacodylate buffer, the fragments were postfixed for 30 min at 4°C with 2% osmium tetroxide in sodium cacodylate buffer, dehydrated through graded ethanol solutions and then processed for embedding in Epon.

In situ *terminal transferase method*

To pinpoint the precise location of DNA, the *in situ* terminal deoxynucleotidyl transferase procedure and its controls were used as previously described (Thiry, 1992a).

Ultrathin sections were incubated for 30 min at 37°C on the surface of the following medium: 20 μM 5 bromo-2-deoxyuridine triphosphate (Sigma, St Louis, USA), 100 mM sodium cacodylate (pH 6.5), 2mM $CoCl_2$, 10 mM ß-mercaptoethanol, 50 μg/ml bovine serum albumin (BSA) and 125 U/ml calf thymus TdT (Boehringer Mannheim, Germany). Sections were again incubated for 30 min at 37°C in the same medium supplemented with 4 μM each of dCTP, dGTP, and dATP (Gibco BRL, Gent, Belgium). This step was indispensable because it has been demonstrated that the anti-BUdR antibody used in this study very weakly detects BUdR on single-stranded tails of DNA (Kitazawa *et al.*, 1989). In some experiments, the sections were first floated for 60 min on a saturated solution of sodium metaperiodate at room temperature. After two rinses in bidistilled water, the various sections were incubated for 30 min in PBS (0.14M NaCl, 6mM Na_2HPO_4, 4mM KH_2PO_4, pH 7.2) containing goat normal serum (GNS) diluted 1/30 and 1% BSA, then rinsed with PBS containing 1% BSA. The next step of the treatment was 4-hr incubation at room temperature with monoclonal anti-BUdR antibody (Becton Dickinson, Mt View, CA) diluted 1/50 in PBS containing 0.2% BSA and GNS diluted 1/50. After washing with PBS containing 1% BSA, the sections were incubated at room temperature for 1 h with goat anti-mouse IgG coupled to colloidal gold (10 nm diameter, Janssen Life Sciences, Beerse, Belgium) diluted 1/40 with PBS (pH 8.2) containing 0.2% BSA. After washing with PBS containing 1% BSA, the sections were rinsed in deionized water. Finally, the 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.

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