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Nuclear Distribution of RNA Polymerase II in Human Oocytes From Antral Follicles: Dynamics Relative to the Transcriptional State and Association With Splicing Factors

Vladimir N. Parfenov,[1] Donna S. Davis,[3] Galina N. Pochukalina,[1] D. Kostyuchek,[2] and Kuruganti G. Murti[3,4]*

[1]Institute of Cytology, Russian Academy of Science, St. Petersburg, Russia
[2]State Medical Academy, St. Petersburg, Russia
[3]Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee 38105
[4]Department of Pathology, University of Tennessee, Memphis, Tennessee 38163

Abstract The intranuclear distribution of two (unphosphorylated and hyperphosphorylated) forms of RNA polymerase II (Pol II) was studied in human oocytes from antral follicles using immunogold labeling/electron microscopy. The distribution of Pol II was analyzed relative to the transcriptional state of the oocyte as well as to the distribution of two splicing factors (snRNPs and SC-35) in the intranuclear entities, namely, interchromatin granule clusters (IGCs), nucleolus-like bodies (NLBs), and perichromatin fibrils (PFs). The results showed that (1) antibodies directed against two forms of Pol II have similar pattern of intranuclear distribution, (2) both Pol II and splicing factors progressively accumulate in IGCs with decrease in the transcriptional activity of the oocyte nucleus, (3) both Pol II and splicing factors localize to PFs, and (4) Pol II is present in the NLBs at all transcriptional states of the oocyte nucleus. These studies confirm earlier proposals that PFs represent a nuclear domain in which RNA transcription/processing are spatially coupled. The accumulation of Pol II and splicing factors in IGCs concomitant with a decrease in the transcriptional activity suggests a coordinated mechanism for the movement of both Pol II and splicing factors from the sites of action to the sites of storage. J. Cell. Biochem. 77:654–665, 2000.

Key words: human oocytes; immunogold labeling; RNA polymerase II; splicing factors

In the last decade, many studies have focused on the molecular aspects of eukaryotic gene expression and identified the mechanisms and the participants [for reviews see Corden, 1993; Moore et al., 1993; Koleske and Young, 1995; Fu, 1995; Dahmus, 1996; Manley and Tacke, 1996; Greenblatt, 1997; Will and Lührmann, 1997]. However, much less is known concerning spatial organization of nuclear components in which the RNA transcription and processing occur. Ultrastructural studies have identified certain morphological entities in the eukaryotic nucleus [Monneron and Bernhard, 1969], and immunocytochemical studies have attempted to delineate the function of these entities. Some of these studies have localized certain splicing factors (small ribonucleoprotein particles or snRNPs) and a non-snRNP factor (SC35) in perichromatin fibrils (PFs) and interchromatin granule clusters (IGCs) of the eukaryotic nucleus [Fakan et al., 1984; Puvion et al., 1984; Spector et al., 1991; Raska et al., 1992; Fakan, 1994]. Additionally, it was found that SC35 may shuttle between PFs and IGCs [Spector, 1996; Huang and Spector, 1996; Misteli and Spector, 1997]. Immunofluorescence studies have found that RNA polymerase II (Pol II) colocalizes with splicing factors in

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*Correspondence to: Dr. K. G. Murti, Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, TN 38105. E-mail: gopal.murti@stjude.org

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actively transcribing nuclei [Bregman et al., 1995; Kim et al., 1997; Zeng et al., 1997; Grande et al., 1997]. From all of these studies emerged proposals that (1) PFs represent the nascent transcripts, (2) IGCS represent areas where storage/assembly of splicing factors occurs, and (3) transcription and processing are spatially coupled.

The objective of our research is to map and define the nuclear components involved in RNA transcription and processing in order to test the above proposals. To this end, we have chosen oocytes as the experimental system because they possess a spectrum of transcriptional activity, from highly active to inert, depending on the developmental stage. In a previous study [Parfenov et al., 1998] using immunoelectron microscopy of human oocytes from antral follicles, we discovered a progressive shift of splicing factors from karyoplasm to IGCs as the transcription activity gradually diminishes. Thus, our studies ascribed a potential storage role for IGCs. In the present study, we focus on the dynamics of association of Pol II with various nuclear components as the oocytes progress from transcriptionally active to inert state. Additionally, we examine the topological relationship between Pol II and splicing factors in the various transcriptional states of oocytes.

MATERIALS AND METHODS

Oocytes

The ovarian fragments containing antral follicles were obtained from biopsy material of 14 women (aged 27–43 years) who underwent surgery for gynecological diseases at the State Medical Academy, St. Petersburg, Russia. Diameter of the antral follicles used varied from 7 to 25 mm. According to a published classification [Tesaric et al., 1983], all of them belong to the group of large antral follicles. The cumulus-enclosed oocytes of the three consecutive stages of development (see below) isolated by puncture of follicle wall were immediately fixed in 3.7% formaldehyde and 0.1% glutaraldehyde in phosphate-buffered saline (PBS) for 1 h.

Antibodies

Rabbit anti-RNA polymerase II polyclonal antibody (RNAP), which reacts with the phosphorylated C-terminal domain (CTD) of the hyperphosphorylated form of the large subunit of RNA polymerase II (POL II-LS) [Kim and Dahmus, 1986; Cadena and Dahmus, 1987], was a gift from Dr. M. Dahmus, University of California, Davis, CA. Monoclonal antibody (Mab), 8 WG16, directed against unphosphorylated form of the CTD of RNA polymerase II [Thompson et al., 1989] was bought from Research Diagnostics, Inc. (Flanders, NJ). Mab against Sm epitope (Y12) of SnRNPs [Lerner et al., 1981] was provided by Dr. J. Steitz (Yale University, New Haven, CT). Mab against the SC35 splicing factor [Fu and Maniatis, 1990] was a gift from Drs. X.-D. Fu and T. Maniatis of Harvard University (Cambridge, MA). The secondary antibodies conjugated with gold were from Electron Microscopy Sciences, Fort Washington, PA.

Immunogold Labeling

Electron microscopy and immunogold labeling were performed as described earlier [Murti et al., 1990; Parfenov et al., 1996; 1998]. After embedding in LR white resin, the oocytes were serially sectioned on a Reichert or Sorvall ultramicrotomes and sections were picked up on nickel grids. To minimize nonspecific binding of antibodies, the grids were floated on PBS containing 0.02 M glycine and Tris-buffered saline (TBS) containing 0.5% fish gelatin (15 min each). The grids were then floated on primary and secondary antibodies diluted with TBS containing 1% fish gelatin for 1.5 h at 37°C. RNAP and 8WG16 antibodies were diluted 1:50 and 1:10, respectively. Y12 was diluted 1:10. Anti-SC35 was used as undiluted culture supernatant. The grids were rinsed, stained with 4% aqueous uranyl acetate, and viewed in a JEOL (Japan Electron Optics Limited) 1200 EXII electron microscope operated at 80 KV. Controls were maintained by omitting the primary antibody from the procedure or by using an irrelevant primary antibody; neither of these controls showed significant labeling. For double-immunogold labeling of oocyte with RNAP and anti SC35 or Y12 antibodies, the sections were sequentially incubated with RNAP (rabbit) antibodies followed by goat anti-rabbit antibodies coupled with large (15-nm) gold particles. After rinsing, the sections were incubated with anti-SC35 or Y12 Mabs followed by goat anti-mouse antibodies coupled with small (5-nm) gold particles. Quantitation of gold particles was done as follows. The gold particles were counted on randomly chosen

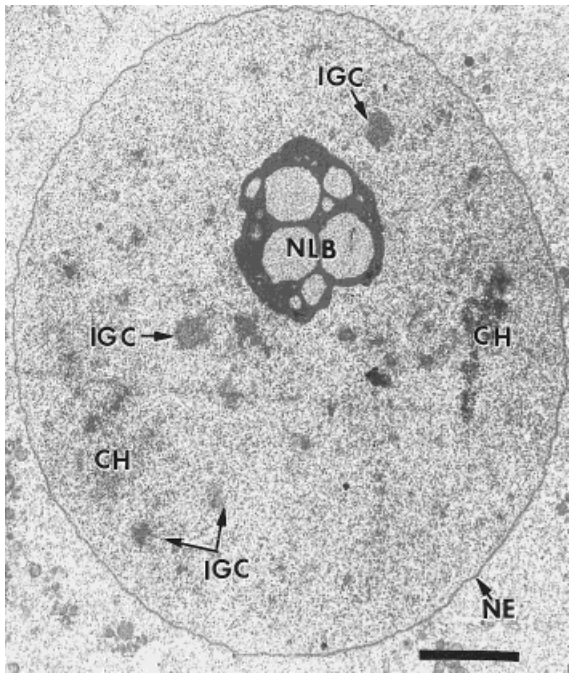

Fig. 1. General nuclear morphology of active-stage human oocyte from antral follicle. NLB, nucleolus-like body; CH, chromatin; IGC, interchromatin granule clusters; NE, nuclear envelope. Scale bar = 2 μm.

fields measuring 17.28 μm^2 in photographic prints at ×20,000 magnification. The counts were performed on seven fields from seven sections per nucleus; we used three oocytes from active and inactive stages.

RESULTS

In our previous studies of the functional organization of oocyte nuclei from human antral follicles, we defined three different stages (i.e., active, intermediate, and inactive) based on the morphology and distribution of intranuclear components and by the analysis of intensity of [5-^{3}H] uridine labeling (transcriptional activity) using electron microscope autoradiography [Parfenov et al., 1989; Gruzova and Parfenov, 1993]. In the present study, we used serial sections of these three groups of oocytes for immunogold labeling with anti-Pol II and anti-splicing factor antibodies.

Active Stage

The nuclear morphology at this stage corresponds to that described earlier [Gruzova and Parfenov, 1993; Parfenov et al., 1989, 1998]. Its general appearance is shown in Figure 1. The central portion of the nucleus is occupied by a vacuolated nucleolus-like body (NLB) composed of homogenous filamentous material [Szöllösi et al., 1991]. Numerous IGCs are randomly distributed in the nucleus as described earlier [Parfenov et al., 1998]. The fine structure of these components corresponds to that described in early literature [Swift, 1969; Monneron and Bernhard, 1969; Fakan and Puvion, 1980]. The karyoplasm of the oocyte at this stage contains diffuse euchromatin, and a few small patches of partly condensed chromatin. PFs associated with chromatin are readily distinguished, and PFs are often found mixed with granules, as was mentioned in early electron microscope studies [for review, see Puvion and Moyne, 1981]. The fibrogranular material containing PFs is connected with large (approximately 40 nm) granules resembling perichromatin granules (PGs) [Daskal, 1981; Puvion and Lange, 1980] and IGCs.

The karyoplasm of this stage oocyte after immunogold labeling with two anti-Pol II probes (RNAP and 8WG16) revealed a similar distribution of the label (Fig. 2A,B). The labeling intensity is also somewhat similar with 35 gold particles per 17.28 μm^2 for RNAP and 48 gold particles for 8WG16 (see Materials and Methods for quantitation procedures). To determine the relationship between the distribution of the transcription and splicing machinery, we performed double immunogold labeling with RNAP antibodies and Y12 and two different-sized gold particles (see Materials and Methods). The results are shown in Fig. 2C. The distribution of Y12 corresponds to that of Pol II, and both are localized on chromatin and predominantly on PFs. We have also performed quantitative double immunogold labeling studies with SC35 versus RNAP and Y12 versus RNAP to determine the relative intensity of labeling. The results showed the following distribution of gold particles: 33 (Y12) : 35 (RNAP), and 11(SC35) : 35 (RNAP).

The IGCs in active stages nuclei, despite their similar morphology, demonstrate different levels of anti-Pol II (RNAP and 8WG16) labeling. Some IGCs accumulate notable amounts of label, whereas others much less or none, but all IGCs demonstrate significant accumulation of SC-35 label. A double labeling study of both of these antibodies is shown in Fig. 3A.

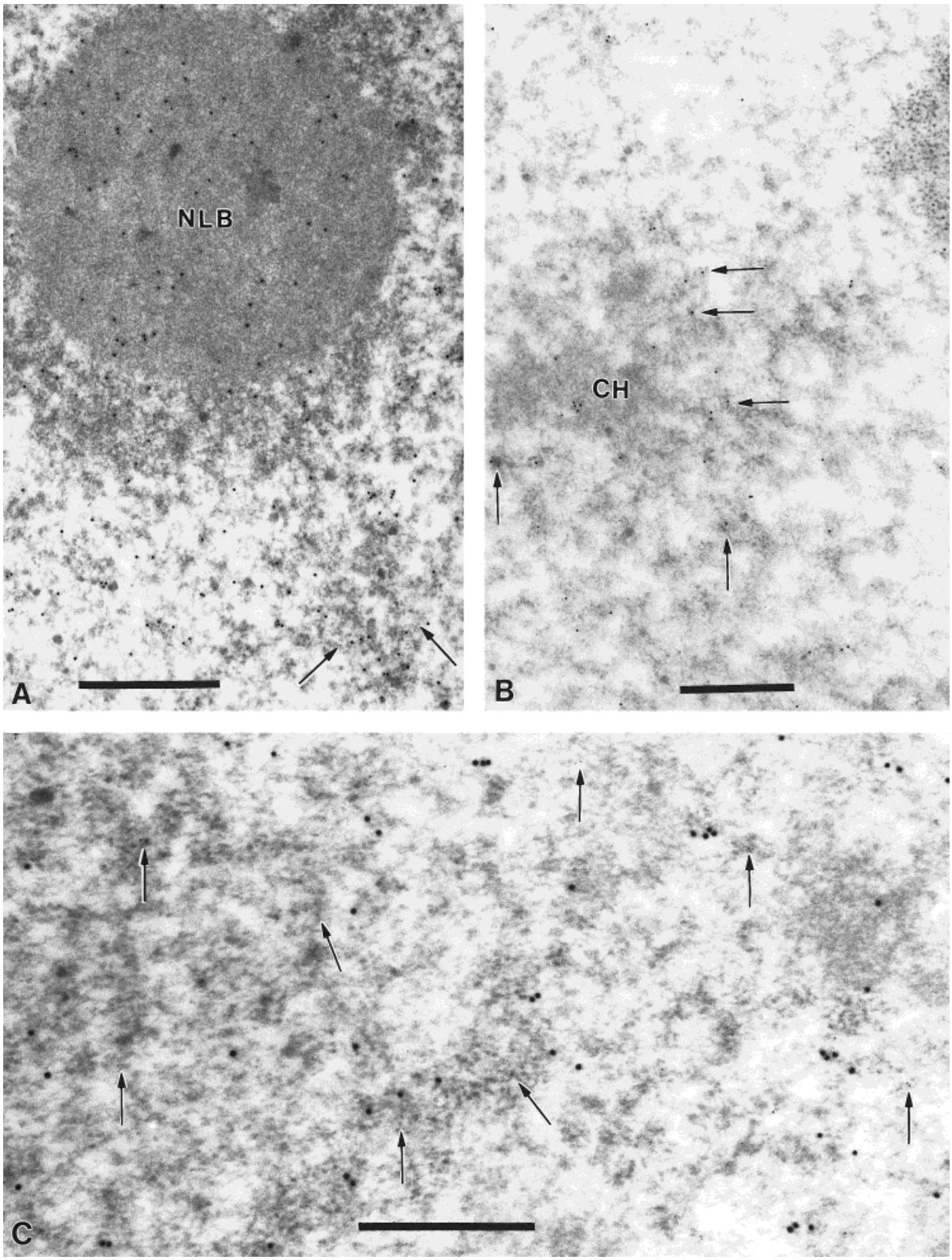

Fig. 2. Immunogold labeling of the active-stage human oocyte with anti-Pol II antibodies [8WG16 and rabbit anti-RNA polymerase II polyclonal antibody (RNAP)] and anti-Sm (Y12). **A:** Labeling with 8WG16. Note the localization of label in NLB (nucleolus-like body) and in clump of perichromatin fibrils (PFs) (arrows). **B:** Labeling with RNAP. Label localizes on PFs (arrows), which are connected with small mass of condensed chromatin (CH). **C:** Double-immunogold labeling of karyoplasm with RNAP and Y12; large gold particles show RNAP labeling and small gold particles denote anti-Sm (Y12) labeling (arrows). Scale bars = 0.5 μm.

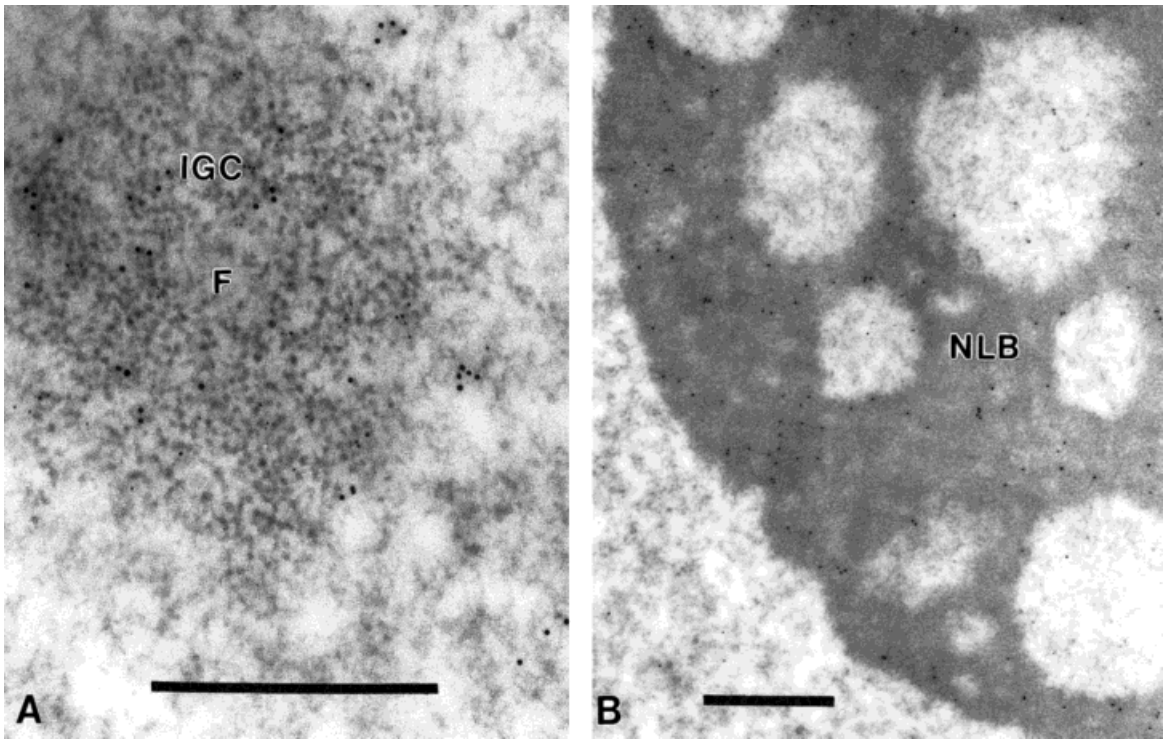

Fig. 3. Immunogold labeling of nuclear structures in active-stage oocyte. **A:** Double-immunogold labeling of interchromatin granule clusters (IGC) with rabbit anti-RNA polymerase II polyclonal antibody (RNAP) and anti-SC35 antibodies. Note the absence of both RNAP and anti-SC35 labeling in the fibrillar (F) region of IGC. **B:** Labeling of nucleolus-like body (NLB) with 8WG16. Scale bars = 0.5 μm.

A striking feature of the present immunogold labeling with anti-Pol II is the accumulation of RNA polymerase II in NLB. Although the NLBs exhibit differences in morphology from active to intermediate to inactive stages, they all show a similar pattern of labeling (Figs. 2A and 3B) with anti-Pol II antibodies. Additionally, the NLBs of all stages failed to label with SC35 and Y12 antibodies, confirming our previous results.

Intermediate Stage

In this stage, the oocyte nuclei exhibit some morphological features that are indicative of the decrease in nuclear transcriptional activity. The chromatin condensation increases, and patches of tightly packed fibrillar material occur in different parts of the nucleus. Some of these patches associate with NLB, reflecting the beginning of the aggregation of the nuclear structures including IGCs around NLB (Fig. 4). This step of intranuclear aggregation is accompanied by the condensation of NLB concomitant with the disappearance of vacuoles. The NLBs of oocyte nuclei at intermediate stage, as in the active stage, are composed of homogenous material of fine filaments. The IGCs of intermediate-stage oocytes appear roughly spherical and larger (Fig. 4). The periphery of condensed chromatin patches is associated with fibrogranular material, which includes mainly PFs and some PGs. This material does not uniformly outline the chromatin patches and is present as irregular clumps at the periphery of chromatin (Fig. 5A). Fibrogranular material containing PFs often extends from perichromatin region into interchromatin zone. Portions of same material in the form of wide tracks are observed in connection with IGCs (Fig. 6B). Because extensions are convoluted and highly branched, it is difficult (even using analysis of serial sections) to conclude whether they are continuous for long distances up to outlying IGCs.

Immunogold labeling of the intermediate stage of oocytes with both anti-Pol II antibodies, RNAP and 8WG16, shows the same labeling pattern. In karyoplasm, the label is pre-

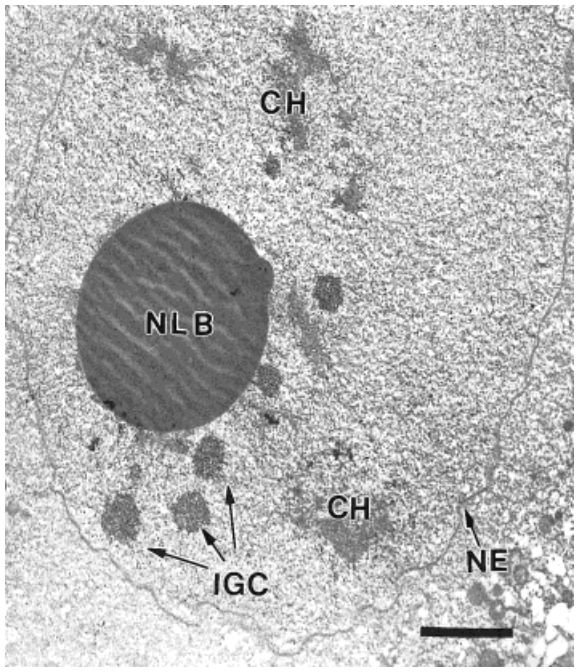

Fig. 4. General morphology of the intermediate-stage oocyte nucleus. Note the condensation of chromatin (CH) and the beginning of aggregation of nuclear entities [CH, interchromatin granule clusters (IGCs)] around the compact nucleolus-like body (NLB). Scale bar = 2 μm.

dominantly associated with PFs in the perichromatin region and fibrogranular extensions in interchromatin space (Fig. 5A,B). The double immunolabeling study shows that label of RNAP has the same pattern of distribution as label of antisplicing factors. In contrast to active stage, the intermediate stage ICGs display a gradual increase of Pol II accumulation. As was evident with RNAP and 8WG16 labeling, all IGCs appear to contain anti-Pol II label (Fig. 5B). However, the IGCs of intermediate stage, as was shown on serial sections, are readily distinguishable by intensity of labeling. Some of them have the amount of label about two to three times higher than others. It should be mentioned that the fibrillar zone of IGCs remains unlabeled with RNAP or 8WG16 antibodies (Fig. 5B,C); the same results were obtained with anti-SC35 in double-labeling experiments (Fig. 5C), confirming our previous data [Parfenov et al., 1998]. The IGCs of intermediate stage display a high level of colocalization frequency of RNAP antibody with anti-SC35 (Fig. 5C).

The NLBs of intermediate-stage oocytes show similar pattern and intensity of RNAP and 8WG16 labeling as to those at active stage. In contrast to IGCs, NLBs of this stage of oocyte development do not include any splicing factors as revealed in double labeling studies (Fig. 6A). This was further confirmed using the technique of serial sections. Pol II and SC35 appear to localize to the narrow rim of IGCs that face the adjacent chromatin (Fig. 5D,E).

Inactive Stage

At this stage, all nuclear structures are gathered around NLBs, the condensed chromatin is connected with the surface of the NLB, and IGCs associated with chromatin are visible in this area of the nucleus (Fig. 7). Our previous autoradiographic studies [Parfenov et al., 1989; Gruzova and Parfenov, 1993] showed that this stage of human oocytes is transcriptionally inert. Immunogold labeling of sections of these oocytes with two anti-Pol II antibodies showed a dramatic decrease in the density of label in the karyoplasm (6/17.28 μm^2) as compared with the active stage (35–48/17.28 μm^2, and a sequestration of label in IGCs and NLBs. In the karyoplasm, the sparse anti-Pol II label mostly localized where small masses of fibrogranular material containing the PFs. Double-labeling studies with antibodies to RNAP and anti-SC35 demonstrated that Pol II and SC35 colocalize in these small masses. In the rest of the karyoplasm, Pol II and SC35 distribute in a diffuse pattern. IGCs of the inactive stage show the same pattern of anti-Pol II labeling (Fig. 7) as that of the intermediate stage. Double-labeling studies with RNAP and SC35 antibodies at this stage revealed that IGCs continue to accumulate Pol II and splicing factors (Fig. 7). The inactive-stage NLBs are labeled with anti-Pol II antibodies with density comparable to the labeling at the intermediate stage, but they remain unlabeled either with Y12 or anti-SC35 (Fig. 7).

DISCUSSION

The immunogold labeling study presented here describes four key observations:

1. Antibodies directed against two forms of Pol II, hyperphosphorylated and unphosphorylated, have similar patterns of nuclear distribution in human oocytes from antral follicles.
2. IGCs show the accumulation of Pol II at all three stages of the oocyte with different

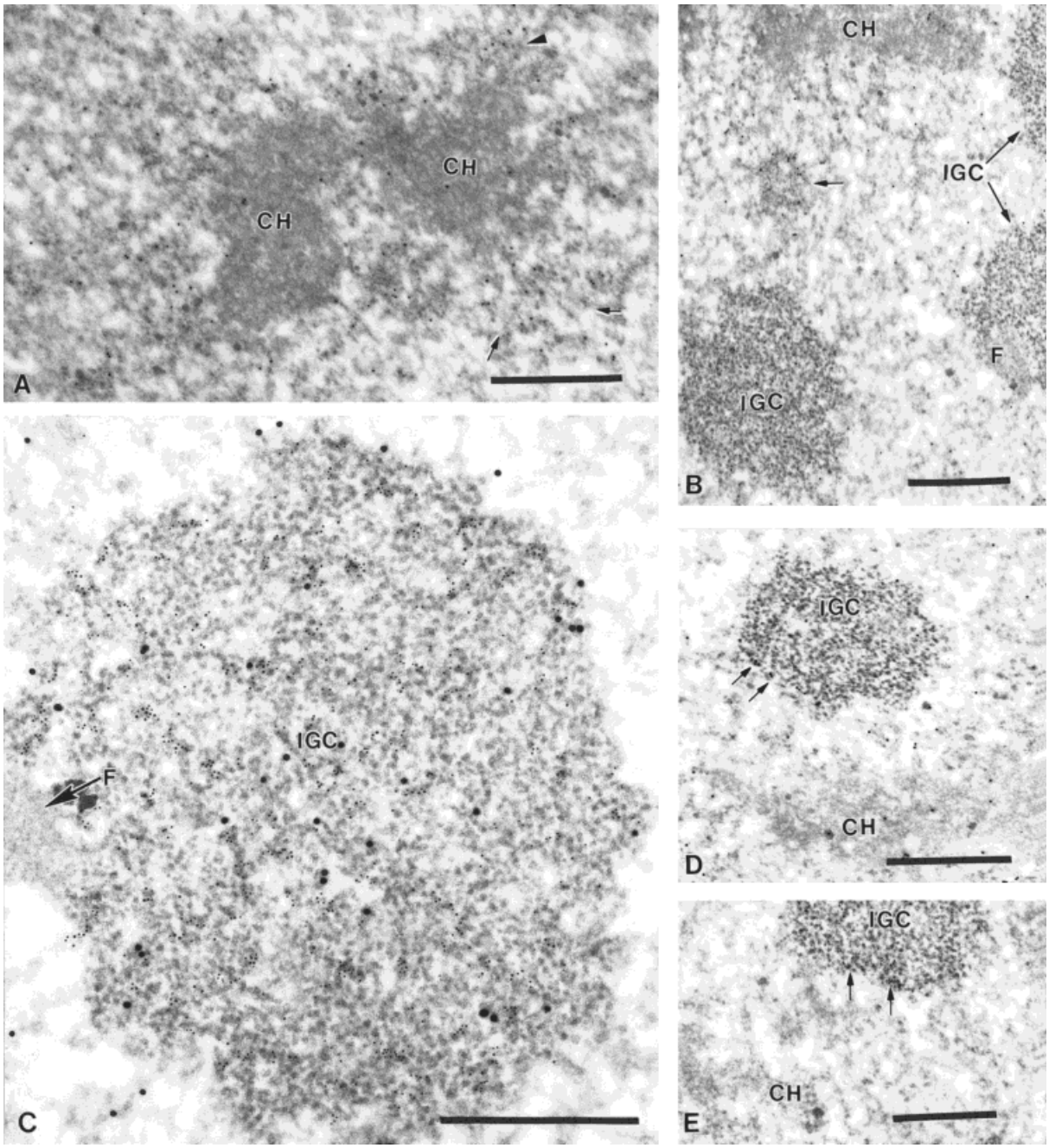

Fig. 5. Immunogold labeling of intermediate-stage oocyte nuclear structures. **A:** Portion of nucleus with a patch of condensed chromatin (CH) after labeling with 8WG16. Note the labeling on CH border (arrowhead) and in connection with clump (arrows) of fibrogranular material containing perichromatin fibrils. **B:** Immunogold labeling of a part of nucleus containing condensed CH, interchromatin granule cluster (IGC), and portions of two IGCs (two arrows) with 8WG16. Note the clump of labeled fibrogranular material (single arrow) between CH and IGC. Note the labeling of three of the IGCs. Also note that the fibrillar zone (F) of IGC remains unlabeled. **C:** Double-immunogold labeling of the IGC with rabbit anti-RNA polymerase II polyclonal antibody and anti-SC35 antibodies. Note the labeling on the granular part but not the fibrillar region of IGC (F, arrow). **D:** Labeling of two serial sections containing IGC in close proximity to CH. Note topologically the same edge of IGC (arrows) predominantly labeled with 8WG16 (D) or anti-SC35 (**E**). Scale bars = 0.5 μm.

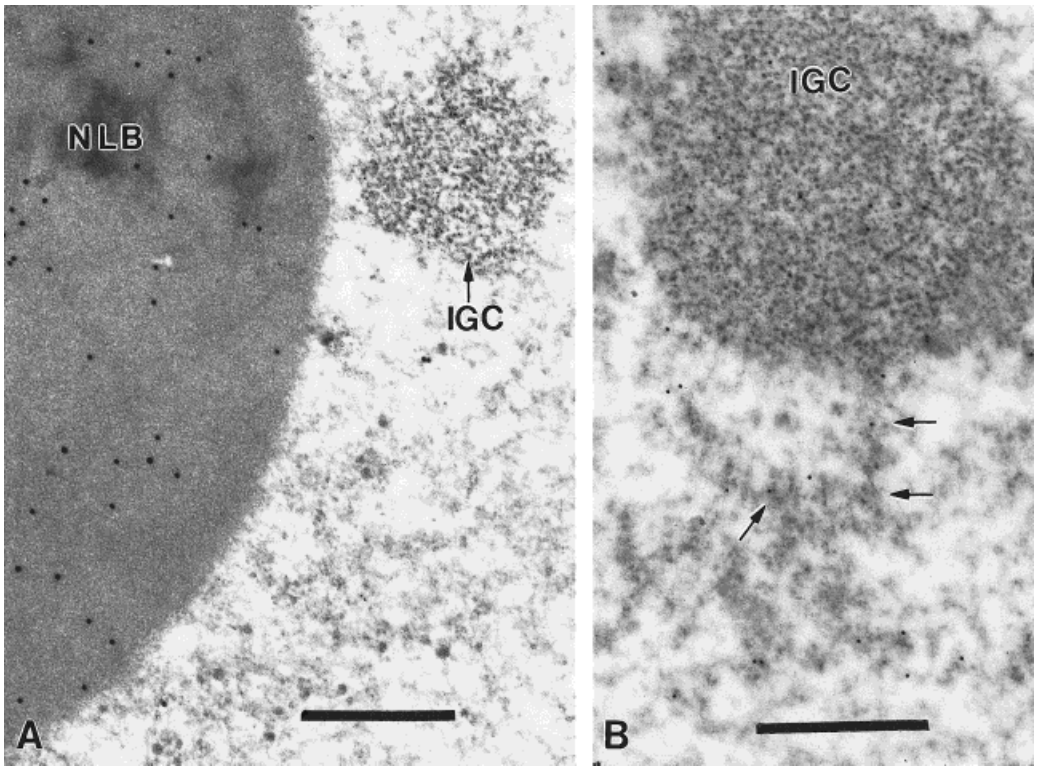

Fig. 6. Different areas of intermediate-stage oocyte nucleus after double-immunogold labeling [rabbit anti-RNA polymerase II polyclonal antibody (RNAP) and anti-SC35] (**A**) and labeling with 8WG16 (**B**). Note that nucleolus-like body (NLB) is labeled only with RNAP (large gold particles), whereas interchromatin granule cluster (IGC) shows the labeling with anti-SC35 (small gold particles). Note that the fibrogranular material labeled with 8WG16 (B) is connected to the IGC (arrows). Scale bars = 0.5 μm.

transcriptional activities. At active stage, there appear to be three populations of IGCs with different levels of anti-Pol II labeling.

3. Pol II is present in NLBs at all three stages of oocyte.
4. The karyoplasmic labeling of Pol II is associated with PFs and localize there with splicing factors.

In our experiments, we used antibodies (RNAP and 8WG16) against two forms of Pol II. It was known that RNAP reacts predominantly with hyperphosphorylated CTD of the large subunit of Pol II [Cadena and Dahmus, 1987], whereas 8WG16 binds to unphosphorylated CTD [Thompson et al., 1989; Bregman et al., 1995; Martillaro et al., 1996]. The unphosphorylated form is believed to take part in the very initial steps of transcription in assembling the preinitiation complex on the promoter [Chesnut et al., 1992; Dahmus, 1996] and the hyperphosphorylated form in the elongation of transcript [Cadena and Dahmus, 1987; Weeks et al., 1993]. Because of these differing roles, we expected different pictures of immunogold labeling with antibodies against these two forms of Pol II. However, in both cases we observed a comparable pattern of labeling. These data also demonstrate the presence of two forms of Pol II in all morphological entities (i.e., PFs, IGCs, NLBs) of human oocyte nuclei. Our observation on the accumulation of Pol II in IGCs is consistent with the earlier immunofluorescence data [Bregman et al., 1995; Mortillaro et al., 1996; Jolly et al., 1999]. Our data on the increasing intensity of Pol II labeling in IGCs with progressive reduction of the nuclear transcriptional activity are in good agreement with the light microscopic evidence showing a significant accumulation of Pol II in nuclear speckles during early G1 and late G2 stages of cell cycle, which are associated with minimal transcription [Bregman et al., 1995] or in cells inactivated by drugs or heat shock [Zeng et al., 1997]. The accumulation of Pol II in IGCs rel-

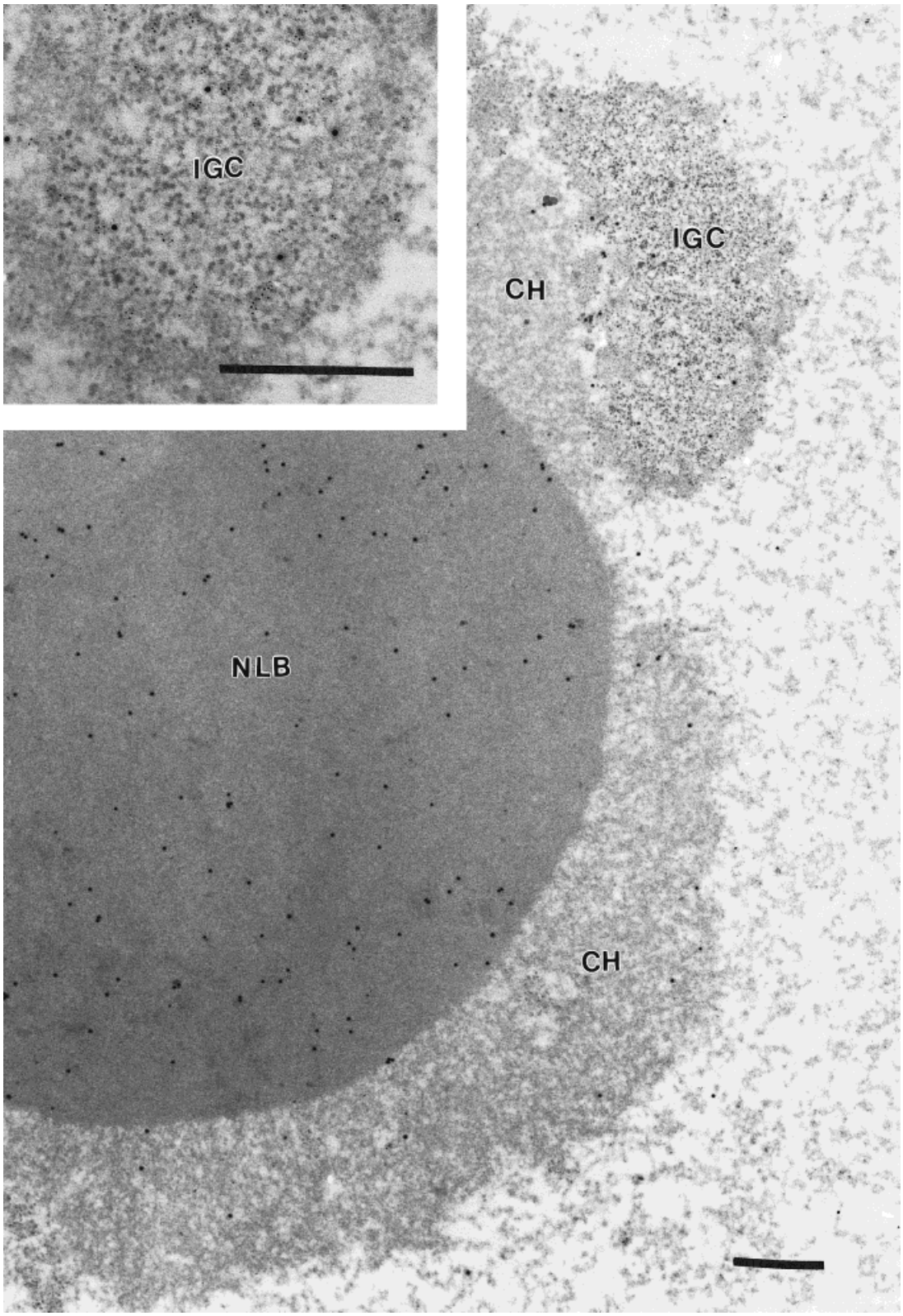

Fig. 7. Double-immunogold labeling of the inactive-stage oocyte with RNAP and anti-SC35. Note the aggregation of nuclear structures [chromatin, interchromatin granule clusters (CH, IGCs)] around the nucleolus-like body (NLB). Note that the NLB exclusively labels with rabbit anti-RNA polymerase II polyclonal antibody (RNAP) (large particles), whereas the IGC labels with both RNAP and anti-SC35 (small gold particles, see **inset**). Also note that the CH and karyoplasm show sparse labeling either with RNAP or anti-SC35. Scale bars = 0.5 μm.

ative to gradual decrease of transcriptional activity may indicate the same storage/recycle role of IGCs for Pol II as it apparently is for splicing factors [for review see Fakan, 1994; Puvion and Puvion-Dutilleul, 1996; Misteli and Spector, 1998]. The accumulation of SnRNPs and SC35 in human oocytes IGCs with reduction of nuclear activity was demonstrated in our previous report [Parfenov et al., 1998]. The storage of Pol II in IGCs leads to the proposal that there exists a shuttling mechanism that transports Pol II to and from IGCs to sites of transcription, as has been proposed for splicing factors [Jiminez-Garcia and Spector, 1993; O'Keefe et al., 1994]. Such a mechanism is attractive because it allows transport of the essential components of transcription-splicing machinery as a single complex [Jolly et al., 1999]. The three populations of IGCs at active stages (intensely labeled, sparsely labeled, and unlabeled) may reflect the dynamic state of the movement of Pol II from sites of storage to sites of transcription.

Our data on anti-Pol II labeling of IGCs at active stage slightly differ from the results obtained with the same Pol II antibodies on T24 (human bladder carcinoma) cells [Cmarko et al., 1999]. In their study, only two groups of IGCs are defined, weakly labeled and unlabeled. The population of IGCs with the substantial labeling described in our study is absent there, and in addition the accumulation of Pol II was found in fibrillar material at the periphery of IGCs. The reason for the difference between the two studies is not clear, but it is possible that it may be because of the different material used—normal human oocytes in our study versus cultured malignant T24 cells in that of the study by Cmarko et al. Both types of cells are morphologically different and may have a different schedule of transcriptional activity. A major difference between the two types of cells in terms of nuclear organization is the absence of interchromatin granule–associated zones [Visa et al., 1993a] in human oocytes at all stages [present study and Parfenov et al., 1989, 1998; Gruzova and Parfenov, 1993] as compared to T24 cells. There are, however, fibrillar zones in the interior of IGCs in oocytes that remained unlabeled either with Pol II or splicing factor antibodies [see Figs. 3A, 5B,C, and Parfenov et al., 1998].

Another notable morphological entity of oocyte nucleus, NLBs, show the anti-Pol II labeling at all three stages, although a little less at the inactive stages. In contrast to IGCs, NLBs of three stages are devoid of splicing factors, either SnRNPs or SC-35. This observation is consistent with results of our previous report [Parfenov et al., 1998], in which NLBs were the only nuclear structure labeled with anti-p80 coilin antibody directed against marker protein of coiled bodies mammalian cells. The reason for the presence of Pol II and coilin in the NLBs remains to be understood.

Concerning the distribution of Pol II in karyoplasm, our data indicate that anti-Pol II labeling is predominantly associated with PFs at active and intermediate stages. At the inactive stage, the sparse Pol II label is diffusely distributed in the karyoplasm. Our data on the association of Pol II with PFs are in good agreement with other immunogold labeling studies [Spector et al., 1993; Cmarko et al., 1999]. These observations, taken together with the evidence of intensive labeling of PFs after H^3-uridine incorporation [for review see Fakan and Puvion, 1980; Puvion and Moyne, 1981], suggest that PFs are the morphological manifestations of nascent transcripts [for review see Fakan, 1994]. We found that at the intermediate stage, the anti-Pol II labeled fibrogranular material, including clumps of PFs, at some distance from perichromatin zones (Fig. 5A,B). Some material in the form of protrusions has been seen in the proximity of IGCs as well (Fig. 6B). Analysis of serial sections gave the impression that the fibrogranular material of the PFs may extend from the perichromatin zone to the IGCs. The potential migration of PFs from perichromatin zone region and their connection with IGCs was repeatedly mentioned in earlier ultrastructural studies [Puvion and Moyne, 1978; Fakan and Puvion, 1980; Visa et al., 1993a, 1993b].

It must be mentioned that most cases of the karyoplasmic localization of Pol II with SC35 or SnRNPs splicing factors at active and intermediate stages were observed in association with PFs. In this respect, our data agree with the observation of Cmarko et al. [1999], who proposed a colocalization of transcription machinery and splicing factors on PFs. Furthermore, other immunogold labeling studies on the localization of splicing factors on PFs [Fakan et al., 1984; Puvion et al., 1984; Kopecny et al., 1991; Spector et al., 1991; Kiseleva et al.,

1994] also suggest that some steps of pre-mRNA processing occur in PFs.

The mechanism of coupling of Pol II with splicing factors and the role of CTD in mediating this process is being intensively investigated. The role of CTD in the functional interaction between Pol II and splicing factors was shown in experiments in which overexpression of CTD blocks the splicing and induces a selective reorganization of splicing factor domains [Du and Warren, 1997]. It is possible that CTD physically binds to splicing factors [Yuryev et al., 1996; Mortillaro et al., 1996; McCraken et al., 1998; Corden and Patturajan, 1997; Kim et al., 1997]. The anchoring of splicing factors on CTD via the CTD-RS domain connection was postulated [Greenleaf, 1993]. It remains unclear whether Pol II molecules actively engaged in transcription are the ones that colocalize with splicing factors. Evidence from transcriptionally inactive mitotic cells demonstrates a noticeable colocalization of Pol II with SnRNPs and some SR (Ser Arg) proteins including SC35 [Kim et al., 1997]. Also, coupling of Pol II disengaged from transcription with SC35 is apparent in IGCs of intermediate and inactive-stage oocytes in our double-labeling experiments (Figs. 5C and 7).

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