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Dynamics of Distribution of Splicing Components Relative to the Transcriptional State of Human Oocytes From Antral Follicles

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Abstract The distribution of two splicing components (snRNP and SC-35) and coilin were studied by immunogold/electron microscopy in human oocytes from antral follicles at different levels of transcriptional activity (i.e., active, intermediate, and inactive). The results showed a decrease of snRNPs and SC-35 in the karyoplasm as the oocytes progress from a transcriptionally active to the inactive state. The main areas of accumulation of both these splicing components in all stages of oocytes appeared to be the interchromatin granule clusters (IGCs). Within the IGCs, the two splicing components seemed to be spatially segregated, with the snRNPs predominantly bound to the fibrillar region, whereas the SC-35 factors are being enriched in the granular zone. The p80 coilin was found only in the nucleolus-like body (NLB), which is present in all three stages of oocytes; no coiled bodies were evident. These data are consistent with the notion that splicing occurs in the karyoplasm and that the splicing components are mobilized from a storage site (IGCs) to the site of action. J. Cell. Biochem. 69:72–80, 1998.

Key words: human oocytes; immunogold labeling; splicing factors; coilin

In the 1990s, considerable attention is being paid to understand the topographical organization of sites of mRNA metabolism in the mammalian nucleus [Spector, 1993]. In this context, some investigators have successfully localized certain nuclear structures containing splicing components. One of these structures, the interchromatin granule clusters (IGCs), was initially discovered by electron microscopy as a morphological entity in the mammalian nuclei [Monneron and Bernhard, 1969; Swift, 1969]. The IGCs were subsequently shown to contain splicing components, including small nuclear ribonucleoprotein particles (snRNPs) [Perraud et al., 1979; Spector et al., 1983; Fakan et al., 1984; Puvion et al., 1984], a factor essential for spliceosome assembly (SC-35) [Spector et al., 1991; Raska et al., 1992] and poly (A) + RNA [Visa et al. 1993b; Huang et al., 1994]. The observation that most sites of active transcription do not colocalize with IGCs led to the proposal that IGCs represent storage sites for splicing components [Fakan et al., 1980; Fakan et al., 1984]. However, the direct demonstration of the association of poly (A) + RNA and pre-mRNAs with IGCs [Moen et al., 1995] suggests that they may have a more direct role in mRNA processing. Another nuclear component implicated in mRNA metabolism are the perichromatin fibrils (PFs), which are found in the vicinity of condensed chromatin [Fakan, 1994]. The PFs were shown to contain hn RNP core proteins [Fakan, et al., 1984] and snRNPS [Puvion et al., 1984]. It is believed that PFs represent a morphological manifestation of hn RNA and that the RNA-processing machinery also associates

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with these structures [Fakan, 1994]. A third structure known to contain splicing components is the coiled body (CB) [Lamond and Carmo-Fonseca, 1993]. The CBs contain the marker protein p80 coilin [Andrade et al., 1991], snRNP components [Raska et al., 1991], and some sn RNAs [Raska et al., 1991; Visa et al., 1993a] but not the SC-35 splicing factor [Spector et al., 1991]. The functional significance of these entities in RNA metabolism remain to be understood.

In this article we correlate the changes in the nuclear distribution of two major splicing components (snRNPs and SC-35) and coilin with changes in the transcriptional activity of human oocytes from antral follicles. In an earlier study we described three different transcriptionally active states (i.e., active, intermediate, and inactive) of these oocytes based on the morphology and electron microscope autoradiography following [^{3}H] uridine incorporation [Parfenov et al., 1989]. By using antibodies specific to snRNPs [Lerner et al., 1981), SC-35 [Fu and Maniatis, 1990], and p-80 coilin [Andrade et al., 1991], we now investigate the distribution of these proteins in the three transcriptional states of the oocyte. The results suggest that (i) snRNPs and SC-35 change their location according to the transcriptional state of the oocyte nucleus, (ii) IGCs represent storage sites for both snRNPs and SC-35 and that these two components occupy distinct regions within IGCs, and (iii) the nucleolus-like bodies (NLBs) previously shown in these oocytes label with the anticoilin antibody but not with antibodies to snRNPs or SC-35.

MATERIALS AND METHODS

Oocytes

The ovarian fragments with antral follicles were obtained from the biopsy material of 16 women (age range 28–45 years) who underwent surgery for gynecological diseases at the State Medical Academy, St. Petersburg, Russia. The cumulus-enclosed oocytes isolated from the antral follicles were immediately fixed in 3.7% formaldehyde and 0.1% glutaraldehyde in phosphate-buffered saline (PBS) for 1 h.

Antibodies

Anticoilin antibody (R288 rabbit antiserum to p80 coilin) was a gift of Dr. E. K. L. Chan [Andrade et al., 1991], The Scripps Research Institute (La Jolla, CA). Monoclonal antibody (Mab) Y12 against the Sm epitope [Lerner et al., 1981] was the courtesy of Dr. J. Steitz (Yale University, New Haven, CT). Mab directed to the SC-35 splicing factor [Fu and Maniatis, 1990] was kindly provided by Drs. X-D Fu and T. Maniatis of Harvard University (Cambridge, MA). The gold-conjugated secondary antibodies were bought from Amersham (Lisle, IL).

Immunogold Labeling

Immunogold labeling and electron microscopy were performed as described previously [Murti et al., 1990; Parfenov et al., 1995, 1996]. Briefly, the oocyte samples were dehydrated and embedded in LR white resin. The blocks were sectioned on a Sorvall ultramicrotome, and sections were picked up on nickel grids. The grids were floated on PBS containing 0.02 M glycine and Tris- buffered saline (TBS) containing 0.5% fish gelatin (15 min each) to minimize nonspecific binding of the antibodies. The grids were floated on primary and secondary antibodies diluted 20- to 50-fold with TBS containing 1% fish gelatin for 1.5 h at 37°C. The grids were rinsed, stained with 4% aqueous uranyl acetate, and viewed in a JEOL 1200 EXII electron microscope operated at 80 kV. Appropriate 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 oocytes with anti-p80-coilin and anti SC-35 antibodies, the sections were sequentially incubated with anti-p80-coilin (rabbit) antibodies followed by goat antirabbit antibodies coupled with large (15-nm) gold particles. After thorough rinsing, the sections were incubated with the anti-SC-35 (monoclonal) antibodies followed by goat antimouse antibodies coupled with small (5-nm) gold particles. The remainder of the procedures is the same as above.

RESULTS

On the basis of the position of nucleus, distribution of nuclear components, and the intensity of [5-^{3}H] uridine labeling, we have classified the oocytes from antral follicles into three stages [Parfenov et al., 1989; Gruzova and Parfenov, 1993], transcriptionally active, intermediate, and inactive. We have used the LR white resin to embed these oocytes and incubated serial sections of these oocytes in various

primary antibodies (anticoilin, anti-SC-35, or anti-Sm) followed by gold-conjugated secondary antibodies. The results with each of these stages are as follows.

Active Stage

Morphologically, the nucleus at this stage mostly contains diffuse euchromatin and a few islands of condensed chromatin. The most notable morphological entities at this stage are clusters of granules (20–35 nm) corresponding to the IGCs described in earlier literature [Swift, 1969; Monneron and Bernhard, 1969; Fakan and Puvion, 1980] and a nucleolus-like body (NLB) [Szöllösi et al., 1991]. The above components with the exception of NLB (NLB is out of the field) can be seen in Figure 1. Immunogold labeling of thin sections of this stage of the oocyte with the anti-Sm (Y12) monoclonal antibody (which recognizes the Sm antigens of snRNPs) revealed a diffuse distribution of the label over the entire nucleus (Fig. 1A). The density of label as measured in the nuclei of three oocytes (7 sections per nucleus) is seven gold particles per μm^2. The label appears mostly associated with chromatin fibrils, including PFs. The label is extremely low in IGCs and in most of the islands of condensed chromatin. Immunogold labeling of the sections with anti-SC-35 (splicing factor) antibodies gave the following results (Fig. 1B). The antibody, unlike the anti-Sm antibody, predominantly labeled the IGCs and, to a much lesser extent, the diffuse chromatin fibrils; on an average there are 1.8 gold particles per μm^2 in the karyoplasm mostly on chromatin fibrils. An interesting finding of this immunogold labeling with the anti-SC-35 antibody is the compartmentalization of SC-35 protein within the IGCs. The IGCs are predominantly granular but contain small distinct spherical fibrillar regions (Fig. 1B inset). As will be shown, in all three stages of oocytes examined (see also Figs. 2–4), the SC-35 protein appears predominantly in the granular region, whereas the snRNPs seem to be associated with the fibrillar regions.

The NLB gave the same labeling pattern in all three stages of the oocytes. Their typical labeling pattern is illustrated in the intermediate and inactive oocytes (Figs. 3 and 4). They do not show labelling with anti-Sm or anti-SC-35 antibodies but label intensely with the anti-p80 coilin antibody. The IGCs in all three stages do not label with the anti-p80 coilin antibodies (Fig. 3).

Intermediate Stage

In this stage, the IGCs and the islands of condensed chromatin accumulate in the center of the oocyte nucleus proximal to the NLB, which makes it easy to observe all nuclear components in a single section. Morphologically, at this stage, increased condensation of the chromatin occurs, suggesting a decrease in the transcriptional activity. Immunogold labeling of this intermediate stage of oocytes with the anti-Sm antibody shows a dramatic decrease of the label over the karyoplasm—an average of 1.4 gold particles per μm^2 or about fivefold decrease in labeling intensity compared with the active stage. The karyoplasm showed the same level of labeling with the anti-SC-35 antibodies. As mentioned earlier, the SC-35 splicing factor seems to be predominantly associated with the granular region of the IGC (Fig. 2A), whereas the snRNPs appear to be mostly associated with the fibrillar region (Fig. 2B).

The ability to visualize all the nuclear components in close proximity and the availability of two primary antibodies (anti-p80 coilin and SC-35) from heterologous hosts prompted us to conduct a double-immunogold labeling study of the nuclear components (i.e. NLB, IGCs, and chromatin). In this study, the large (15-nm) gold particles signify the anti-p80 coilin labeling, whereas the small (5-nm) gold particles denote the anti-SC-35 labeling. The results illustrated in Figure 3 show an intense labeling of NLB with the anti-p80 coilin (Fig. 3A, B) with little or no labeling of the IGC or chromatin in the same field (see large gold particle distribution in Fig. 1A). The anti-SC-35 antibody, on the other hand, labeled exclusively the IGC (see small gold particle distribution in Fig. 3A and at high magnification in Fig. 3C) in the granular region (Fig. 3C); the condensed chromatin was not labeled by this antibody.

Inactive Stage

At this stage, the condensed chromatin associates with the periphery of the NLB, and the IGC move closer to this area. The IGCs are much bigger than the earlier stages, measuring up to 10 µm. As per our previous studies [Parfenov et al., 1989; Gruzova and Parfenov, 1993], this stage of oocyte is transcriptionally inert. When sections of these oocytes were labeled with anti-Sm, anti-SC-35, and anti-p80 coilin antibodies, the following results are ob-

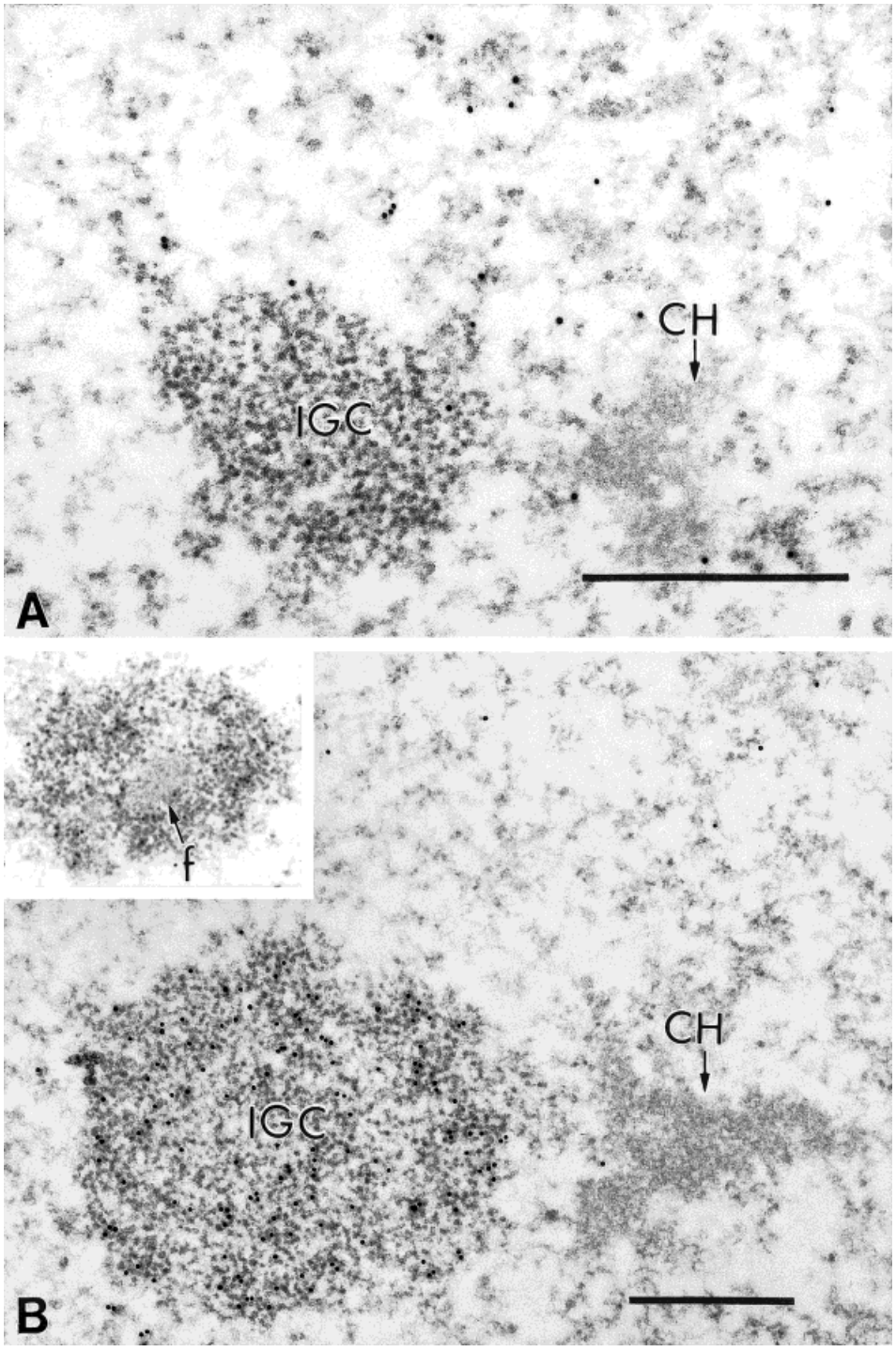

Fig. 1. Immunogold labeling of the active stage human oocyte with anti-Sm (Y12) and anti-SC-35 (splicing factor) antibodies. **A:** The labeling with the anti-Sm antibody. Note the distribution of the gold particles over the karyoplasm. **B:** The labeling with the anti-SC-35 antibody. Note the intense labeling of the granular region of IGC with this antibody and sparse labeling of the karyoplasm, and the absence of the label over the fibrillar (f) zone (inset). Scale bar in this and the subsequent figures = 0.5 μm. IGCs, interchromatin granule clusters; CH, condensed chromatin.

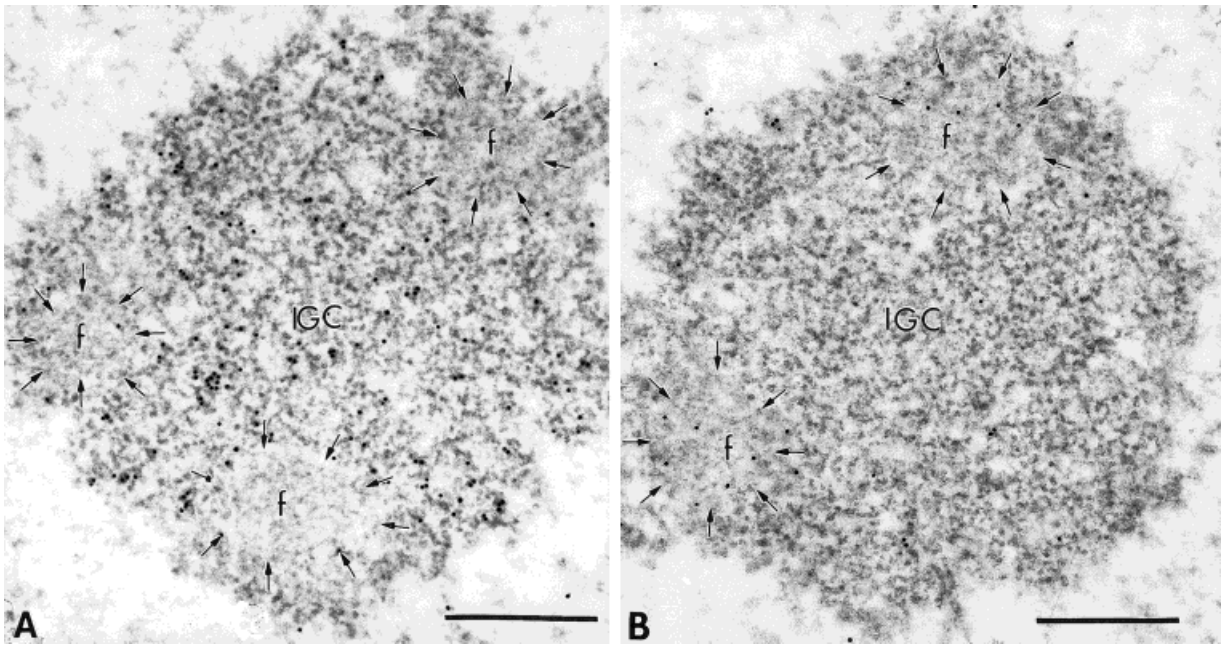

Fig. 2. Immunogold labeling of the IGCs in the intermediate stage of oocytes with the anti-SC-35 (**A**) and anti-Sm (**B**) antibodies. Note the labeling of the anti-SC-35 antibody (A) on the granular region but not the fibrillar region (region marked by arrows). The anti-Sm antibody, on the other hand, predominantly labels the fibrillar region (B).

tained. Most of the karyoplasm is free of SC-35 or snRNP, and the anti-p80 coilin antibody exclusively labeled the NLB (data not shown). As in the previous stage, the label due to anti-Sm antibody is seen over the fibrillar region (Fig. 4A) and the SC-35 antibody over the granular region of IGCs (Fig. 4B); the NLB remains unlabeled with either of these antibodies (Figs. 4A, B).

DISCUSSION

The research presented here contains three key observations: (i) The density of the karyoplasmic labeling of the two splicing components (snRNP and SC35) progressively decrease with the reduction in the transcriptional activity of the oocyte; (ii) the major site of accumulation of snRNPs and SC-35 is the IGCS in which these two components occupy distinct regions, SC-35 in the granular zone and snRNPs in the fibrous zone; and (iii) the only coilin-containing structure detected in all three stages of the oocyte is the NLB, and it does not appear to contain either snRNPs or SC-35.

The progressive decrease of the splicing components in the karyoplasm with a decrease in the transcriptional activity is consistent with the notion that mRNA processing occurs in the karyoplasm [Fakan et al., 1984; Spector, 1993] and that splicing components move from a storage compartment to the site of activity [Huang and Spector, 1996; Spector, 1996]. The storage compartment in the human oocytes could be the IGCs, although we have not directly demonstrated that the progressive loss of label of splicing components in karyoplasm is commensurate with the gain of label in the IGCs. We showed earlier that IGCs increase in size as the oocytes become transcriptionally inactive [Parfenov et al., 1989]. An intriguing finding in this context is the difference in the abundance between the two splicing components in the karyoplasm of active stage oocytes. At this stage, only a small amount of SC-35 appears to associate with the active chromatin in the karyoplasm, although IGCs contain massive quantities of this splicing factor. This is not the case with snRNPs because they appear to be most abundant in the active karyoplasm and least abundant in the inactive karyoplasm. The discrepancy with SC-35 can be explained if it is assumed that the function of SC-35 is transient and that it shuttles back quickly to the storage compartment, i.e., the IGC. It has been suggested that SC-35 functions in the initial step of splicing, the assembly of a spliceosome complex, and that it is not involved in splicing per se [Fu and Maniatis, 1990; Fu, 1993; Manley

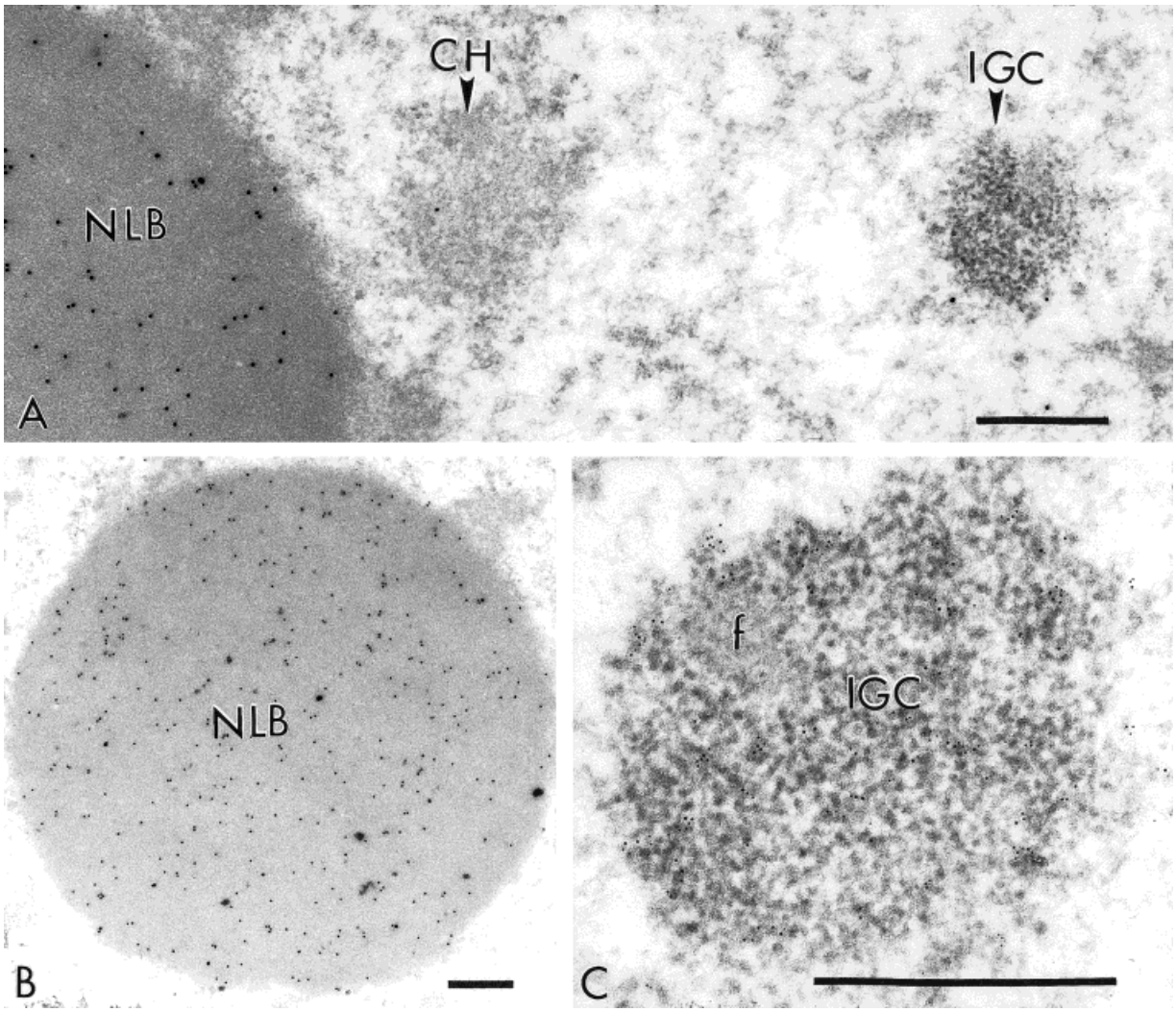

Fig. 3. Double-immunogold labeling of the intermediate stage oocyte with the anti-p80 coilin and anti-SC-35 antibodies. In this figure, the large gold particles demonstrate anti-p80 coilin labeling, whereas the small gold particles show the anti-SC-35 antibody labeling. In (**A**) the portion of the oocyte nucleus illustrated contains a nucleolus-like antibody (NLB), condensed chromatin, and an IGC. The NLB is labeled only with the anti-p80 coilin antibody (also see **B** for the entire NLB), whereas the IGC is labeled only with the anti-SC-35 antibody. The 5-nm gold particles are too small to be seen in the IGC shown in (A) but can be readily seen in a highly magnified IGC shown in (**C**). Note the total absence of large gold particles (coilin) from the IGC and the labeling of the granular region of IGC with the anti-SC-35 antibody (C). Also note the absence of the anti-SC-35 labeling from the fibrillar (f) component of IGC (C).

and Tacke, 1996]. Therefore, it is possible that continued presence of SC-35 in the karyoplasm is not required for splicing. The shuttling of the splicing components from storage sites to active sites and back could be achieved by the phosphorylation/dephosphorylation of these proteins [Colwill et al., 1996; Misteli and Spector, 1997]. It is noteworthy in this context that the SC-35 antibody recognizes a phosphoepitope shared between many SR proteins [Fu et al., 1992] and that the observed labeling pattern was due to the distribution of the phosphorylated form of these proteins.

The reason for the spatial segregation of the two splicing components within the IGCs remains unclear. What is the identity of the fibrils within the IGCs? Are they related to the PF in the karyoplasm? To address these questions, we performed a preliminary immunogold labeling study of oocytes using an anti-DNA antibody which detects the double-stranded DNA [Murti et al., 1990]. The antibody failed to label the fibrillar region of IGCs but labeled the fibrils of condensed chromatin adjacent to the IGCs [Parfenov and Murti, unpublished data]. This study suggests (but does not establish)

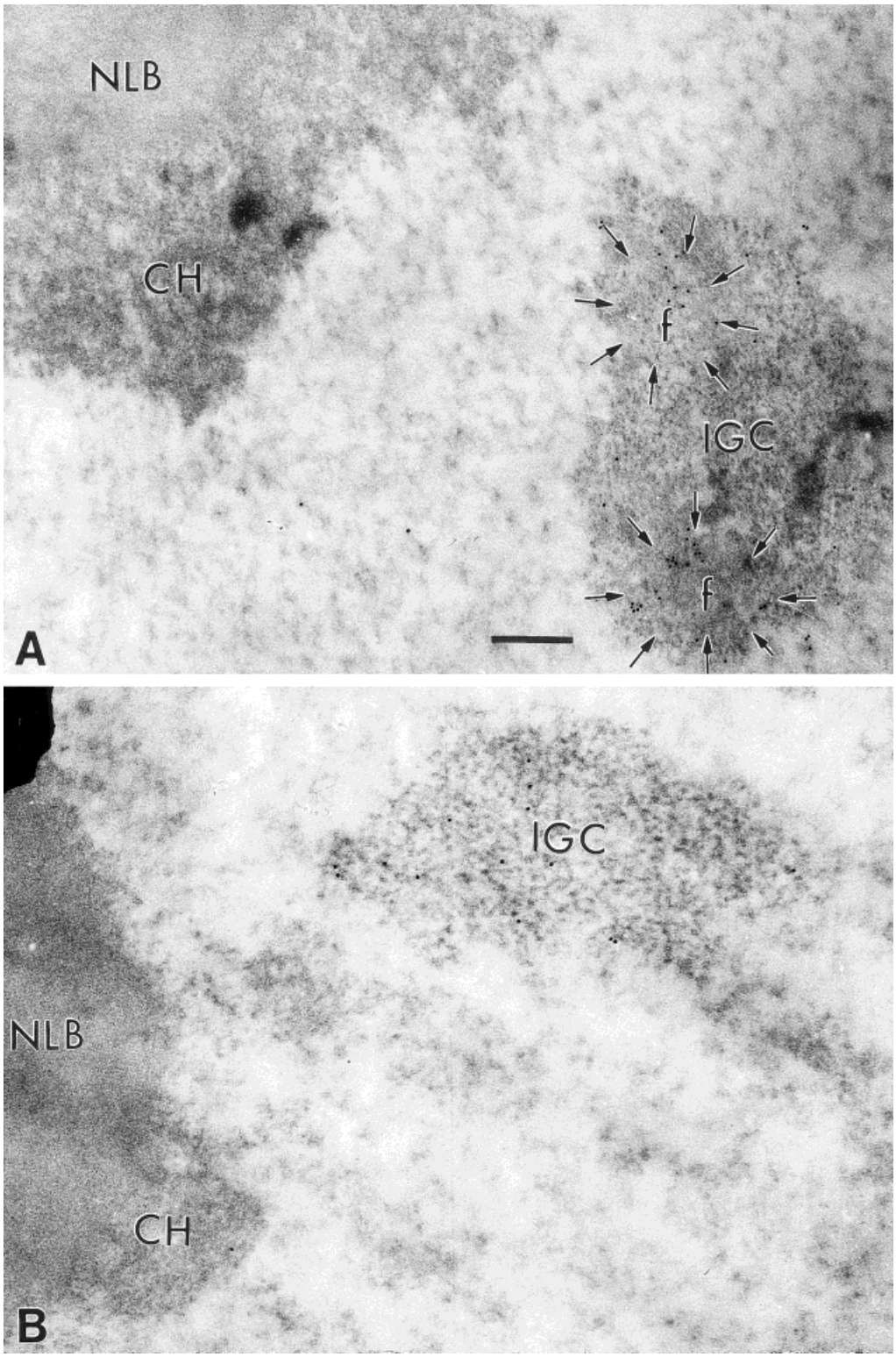

Fig. 4. Immunogold labeling of the inactive stage of the oocyte with anti-Sm (**A**) and anti SC-35 antibodies (**B**). Note the association of condensed chromatin (CH) with the NLB and the close proximity of IGCs to NLB. The label due to the snRNPs is mostly within the fibrillar region (f, outlined by arrows), and that due to SC-35 is in the granular region of IGC (B). Also note that karyoplasm shows little or no label with either antibody.

that the fibrillar zone in IGCs is not DNA. It is possible that this zone contains RNA. It was suggested that IGCs are involved in intron degradation [Raska, 1995]. If it is so, then the fibrillar zone of IGCs may contain degrading introns. In any case, more studies, including in situ hybridization studies are required to address this question.

Our studies suggest that the only structures in the human oocyte karyoplasm that label with both anti-snRNP antibodies and anti-SC-35 antibodies are the IGCs and that the only structures that label with the anti-p80 coilin antibodies are the NLBs. These data differ from the results of earlier studies performed with the oocytes from antral follicles of rats and pigs [Kopecny et al., 1996, 1996a] in which the NLBs were suggested to contain all three (snRNPs, SC-35, and coilin) components. In these studies, the anti-p80 coilin labeling was lower in NLBs than in our study, and the IGCs were not observed. The reason for the differences between our study and that of Kopecny et al. is not clear. Because both studies used (apparently) the same antibodies and comparable immunolabeling procedures, it is to be assumed that the difference lies in the material. For example, it is possible that the SC-35 and snRNP epitopes may be masked in the NLB of human oocytes compared with the IGCs; it may be a reverse situation with other mammals. Alternatively, the differences may reflect true differences in the organization of splicing components between humans and other mammals.

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REFERENCES

Andrade LEC, Chan EKL, Raska T, Peebles CL, Roos G, Tan EM (1991): Human autoantibody to a novel protein of the nuclear coiled body: Immunological characterization and cDNA cloning of p80-coilin. J Exp Med 173:1407–1419.

Colwill KT, Pawson B, Andrews J, Prasad JL, Manley JL, Bell JC, Duncan PI (1996): The CIk/sty protein kinase phosphorylates SR splicing factors and regulates their intranuclear distribution. EMBO J 15:265–275.

Fakan S (1994): Perichromatin fibrils are in situ forms of nascent transcripts. Trends Cell Biol 4:86–90.

Fakan S, Puvion E (1980): The ultrastructural visualization of nucleolar and extranucleolar RNA synthesis and distribution. Int Rev Cytol 65:255–299.

Fakan S, Leser G, Martin TE (1984): Ultrastructural distribution of nuclear ribonucleoproteins as visualized by immunocytochemistry on thin sections. J Cell Biol 98:358–363.

Fu XD (1993): Specific commitment of different pre-mRNAs to splicing by single SR proteins. Nature 365:82–85.

Fu XD, Maniatis T (1990): Factor required for mammalian spliceosome assembly is localized to discrete regions in the nucleus. Nature 343:437–441.

Fu XD, Mayeda A, Maniatis T, Krainer AR (1992): General splicing factors SF2 and SC35 have equivalent activities in vitro, and both affect alternative 5 and 3 splice site selection. Proc Natl Acad Sci USA 89:11224–11228.

Gruzova MN, Parfenov VN (1993): Karyosphere in oogenesis and intranuclear morphogenesis. Int Rev Cytol 144: 1–52.

Huang S, Spector DL (1996): Intron-dependent recruitment of pre-mRNA splicing factors to sites of transcription. J Cell Biol 133:719–732.

Huang S, Deerinck TJ, Ellisman MH, Spector DL (1994): In vivo analysis of the stability and transport of nuclear poly (A) RNA. J Cell Biol 126:877–899.

Kope_ny V, Biggiogera M, Laurincik J, Pivko J, Grafenaw P, Martin TE, Fu XD, Fakan S (1996a): Fine structural cytochemical and immunocytochemical analysis of nucleic acids and ribonucleoprotein distribution in nucleic of pig oocytes and early preimplantation embryos. Chromosoma 104:561–574.

Kope_ny V, Landa V, Malatesta M, Martin TE, Fakan S (1996): Immunoelectron microscope analyses of rat germinal vesicle-stage oocyte nucleolus-like bodies. Reprod Nutr Dev 36:667–679.

Lamond AI, Carmo-Fonseca M (1993): The coiled body. Trends Cell Biol 3:198–204.

Lerner EA, Lerner MR, Janeway CA, Steitz J (1981): Monoclonal antibodies to nucleic acid-containing cellular constituents: Probes for molecular biology and autoimmune diseases. Proc Natl Acad Sci USA 78:2737–2741.

Manley JL, Tacke R (1996): SR proteins and splicing control. Genes Dev 10:1569–1579.

Misteli T, Spector DL (1997): Protein phosphorylation and the nuclear organization of pre-mRNA splicing. Trends Cell Biol 7:135–138.

Moen PhT, Smith KP, Lawrence JB (1995): Compartmentalization of special pre-mRNA metabolism: An emerging view. Hum Mol Genet 4:1779–1789.

Monneron A, Bernhard W (1969): Fine structural organization of the interphase nucleus in some mammalian cells. J Ultrastruct Res 27:266–288.

Murti KG, Davis DS, Kitchingman GR (1990): Localization of adenovirus-encode DNA replication proteins in the nucleus by immunogold electron microscopy. J Gen Virol 71:2847–2857.

Parfenov V, Potchukalina G, Dudina L, Kostyuchek D, Gruzova M (1989): Human antral follicles: Oocyte nucleus and the karyosphere formation (electron microscopic and autoradiographic data). Gamete Res 22:219–231.

Parfenov V, Davis DS, Potchukalina G, Sample CE, Bugaeva EA, Murti KG (1995): Nuclear actin filaments and their topological changes in frog oocytes. Exp Cell Res 217:385–394.

Parfenov V, Davis DS, Potchukalina G, Sample CE, Murti KG (1996): Nuclear bodies of stage 6 oocytes of Rana temporaria contain nucleolar and coiled body proteins. Exp Cell Res 228:229–236.

Perraud M, Gioud M, Monier JC (1979): Intranuclear structures recognized by auto antibodies against ribonucleoproteins: Study on monkey kidney cells in culture using immunofluorescent techniques and immunoelectron microscopy. Annu Immunol 130:635–647.

Puvion F, Viron EA, Assens C, Leduc EH, Jeanteur P (1984): Immunocytochemical identification of nuclear structures containing snSNPs in isolated rat liver cells. J Ultrastruct Res 87:180–189.

Raska I (1995): Nuclear ultrastructures associated with the RNA synthesis and processing. J Cell Biochem 59:11–26.

Raska I, Andrade LEC, Ochs RL, Chan EKL, Chang CM, Roos G, Tan EM (1991): Immunological and ultrastructural studies of the nuclear coiled body with autoimmune antibodies. Exp Cell Res 195:27–37.

Raska I, Dundr M, Koberna K (1992): Structure-function subcompartments of the mammalian cell nucleus as revealed by electron microscopic affinity cytochemistry. Cell Biol Int Rep 16:771–789.

Spector DL (1993): Macromolecular domains within the cell nucleus. Annu Rev Cell Biol 9:265–315.

Spector DL (1996): Nuclear organization and gene expression. Exp Cell Res 229:189–197.

Spector DL, Schrier WH, Busch H (1983): Immunoelectron microscopic localization of snRNPs. Biol Cell 49:1–10.

Spector DL, Fu X-D, Manitais T (1991): Associations between distinct pre-mRNA splicing components and the cell nucleus. EMBO J 10:3467–3481.

Swift H (1969): Studies on nuclear fine structure. Brookhaven Symp Biol 12:134–152.

Szöllösi NS, Debey P, Szöllösi D, Rime H, Vautier G (1991): Chromatin behavior under influence of puromycin and 6-DAMP at different stages of mouse oocyte maturation. Chromosoma 100:339–354.

Visa N, Puvion-Dutilleul F, Bachellerie JP, Puvion E (1993a): Intranuclear distribution of U1 and U_2 snRNAs as visualized by high resolution in situ hybridization: Revelation of a novel compartment contain U1 but not U2 snRNA in Hela cells. Eur J Cell Biol 60:308–321.

Visa N, Puvion-Dutilleul F, Harper F, Bachellerie JP, Puvion E (1993b): Intranuclear distribution of poly(A) RNA determined by electron microscope in situ hybridization. Exp Cell Res 208:19–34.