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Short communication

Interchromatin granule clusters in vitellogenic oocytes of the fleshfly, *Sarcophaga* sp.

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Abstract: Insect oocyte nuclei contain different extrachromosomal nuclear bodies including Cajal bodies and interchromatin granule clusters (IGCs). In the present study, we describe IGC equivalents in the vitellogenic oocytes of the fleshfly, *Sarcophaga* sp. These structures were found to consist of 20-40-nm granules and also include the fibrillar areas of high and low electron density. Immunogold labeling electron microscopy revealed IGC marker protein SC35, Sm proteins, and trimethylguanosine cap of small nuclear (sn) RNAs in these bodies. Antibody against the non-phosphorylated RNA polymerase II selectively labeled the fibrillar areas of low electron density located inside the IGCs.

Key words: Insects - *Sarcophaga* - Oocyte nucleus - Nuclear bodies - Interchromatin granule clusters - Immunoelectron microscopy

Introduction

Interchromatin granule clusters (IGCs) are nuclear bodies (NBs) essentially involved in storage/recycling of pre-mRNA splicing factors including snRNPs and SR-proteins (see [1] for a review), and can be diagnostically revealed with antibodies against the non-snRNP splicing factor SC35 [2,3].

Insect oocytes contain various NBs of different morphology [4]. Their nature is still poorly known. In several species, however, certain oocyte NBs were identified as the counterparts of Cajal bodies (CBs) [5,6,7,8,9,10] and IGCs [5,7,11,12].

Here, we present the results of the immunogold labeling study on *Sarcophaga* vitellogenic oocyte NBs and described oocyte IGC counterparts in this organism.

Material and methods

Specimens of *Sarcophaga* sp. were collected in the village of Toksovo (Leningrad region, Russia) in June. Ovarioles were prepared for immunogold labeling electron microscopy as described [5]. Primary antibodies included the following monoclones: αSC35 against the non-snRNP splicing factor SC35 [2], Y12 against symmetrical dimethylarginines of several Sm proteins [13,14], K121 against the trimethylguanosine cap of snRNAs [15], and 8WG16 against the non-phosphorylated C-terminal domain of RNA polymerase II [16]. Secondary antibodies were goat anti-mouse IgG conjugated with colloidal gold particles of 10 nm (BBInternational).

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Results and discussion

The nucleus of *Sacrophaga* vitellogenic oocytes contains many NBs of various size (Fig. 1). The largest NB is tightly attached to the karyosome [17] like in fruitflies [10,18] and the housefly [4]. In *Drosophila* oocytes, this NB was undoubtedly identified as a CB [10].

Smaller *Sarcophaga* oocyte NBs include irregularly-shaped structures containing 20-40-nm granules and thin fibrils often organized in distinct fibrillar areas of different electron density (Figs 2-5). Immunogold labeling electron microscopy with αSC35 antibody revealed no labeling of the CB but smaller fibrogranular NBs were labeled (Fig. 2). Anti-SC35 labeling was not limited to the granular material of these NBs; some fibrillar zones of higher electron density also cross-reacted with αSC35 antibody (Fig. 2). Anti-snRNP antibodies (Y12 and K121) also labeled the fibrogranular NBs (Figs 3 and 4), but the labeling density was less considerable. In our opinion, the labeling of an NB with anti-snRNP, and, additionally, with anti-SC35 antibodies, allows to identify it as an IGC [7,11]. Anti-RNA polymerase II antibody 8WG16 decorated only the fibrillar areas of low electron density (Fig. 5).

Sarcophaga oocyte IGCs morphologically look similar to those described in the oocytes of the meal-

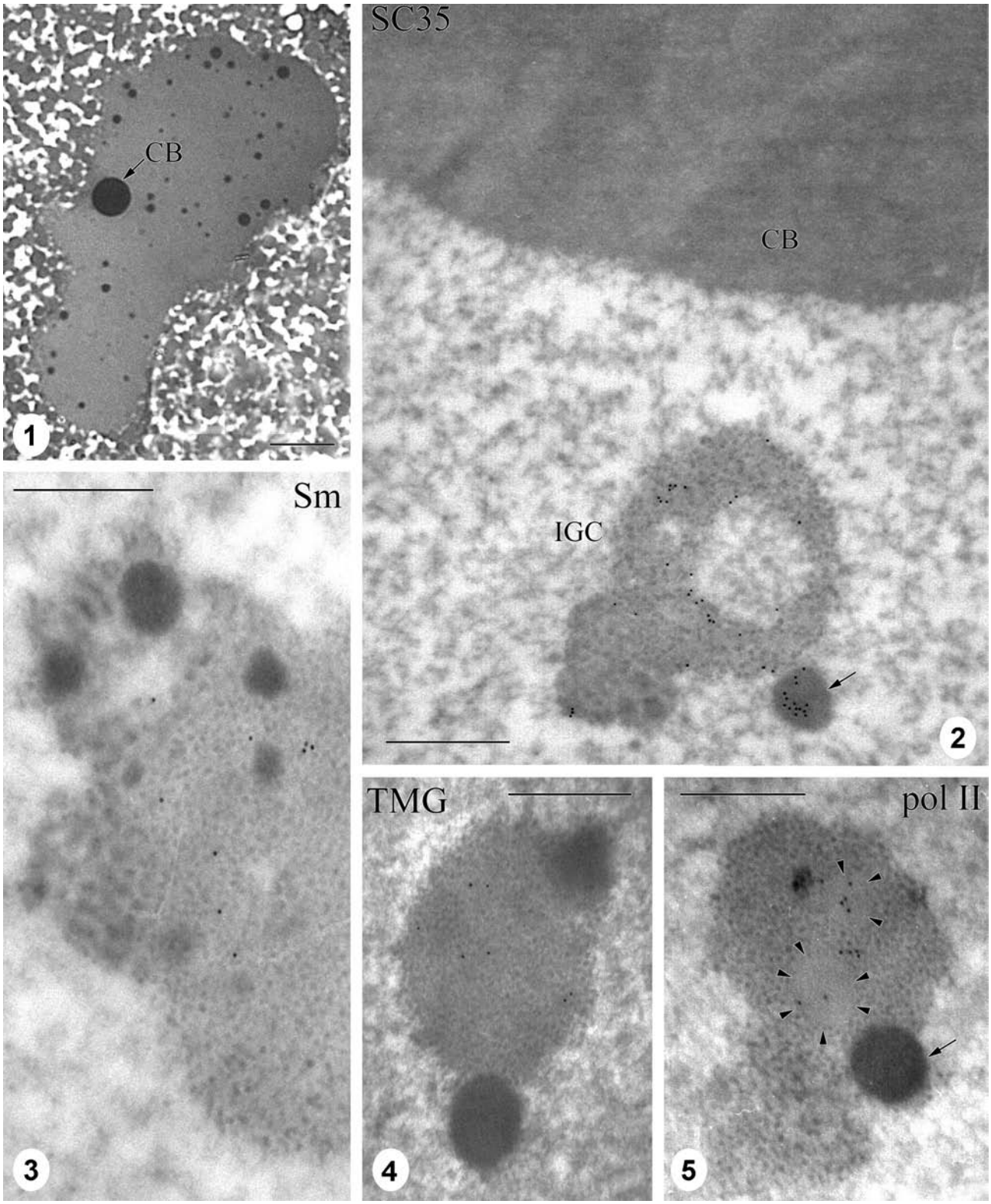

Fig. 1. A semithin section of the nucleus of a *Sarcophaga* vitellogenic oocyte. The largest nuclear body is the Cajal body (CB). Bar = 20 μm.
Fig. 2. *Sarcophaga* oocyte nuclear bodies after immunogold labeling with SC35 antibody. Cajal body (CB) is not labeled whereas the interchromatin granule cluster (IGC) is labeled with this antibody. Arrow indicates an electron dense fibrillar area of IGC. Bar = 0.5 μm.
Figs 3-5. Interchromatin granule clusters of *Sarcophaga* oocytes after immunogold labeling with antibodies Y12 (Fig. 3), K121 (Fig. 4), and 8WG16 (Fig. 5). Note, that anti-RNA polymerase II antibody 8WG16 decorates the fibrillar areas of low electron density (arrowheads); denser fibrillar area (arrow) and the granular material remain unlabeled. Bars = 0.5 μm.

worm, *Tenebrio molitor* [7] and the house cricket, *Acheta domesticus* [12]. *A. domesticus* oocyte IGCs also display prominent fibrillar areas that accumulate RNA polymerase II, especially in oocytes treated with transcription inhibitors DRB [12] or actynomycin D [19]. Taking into account the reduction of transcription rate in dipteran oocyte nuclei [4, 18], one may conclude that oocyte IGCs in these organisms are stores for the factors disengaged from transcription and splicing cycles, as it was predicted earlier for insect oocyte CBs [5,9,11].

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