FOLIA HISTOCHEMICA
ET CYTOBIOLOGICA
Vol. 47, No. 1, 2009
pp. 123-126

Short communication

Factors related to RNA polymerase II transcription are localized in interchromatin granule clusters of *Panorpa communis* oocytes

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Abstract. Diplotene oocyte nucleus of the scorpionfly *Panorpa communis* is transcriptionally silent and contains numerous nuclear bodies including interchromatin granule clusters (IGCs). The latter consist of the granules of 30-50 nm in diameter and contain IGC marker protein SC35 as well as RNA polymerase II. In this study, we also localized in *P. communis* oocyte IGCs the transcription coactivators CBP/p300, TATA-binding protein (TBP) which is a component of the basal transcription factor TFIID and the basal transcription factor TFIIH. We belive that IGCs in transcriptionally inert *P. communis* oocytes are storage sites for the components of RNA polymerase II holoenzyme and other factors of RNA pol II transcription.

Key words: insects, *Panorpa communis*, oocyte nucleus, interchromatin granule clusters, immunoelectron microscopy, RNA polymerase II, transcription factors.

Introduction

Interchromatin granules clusters (IGCs) also referred to as 'speckles' together with Cajal bodies (CB) and nucleoli are considered to be universal extrachromosomal nuclear domains for different cell types [1]. The nature of some characteristic inclusions observed in oocytes of different insects as the CBs are established now [2-5] whereas until recently little is known about insect oocyte IGC structure and functions. Insect oocyte IGCs have been described only in a beetle [6,7], the house cricket [8], a fleshfly [9] and some bugs [10]. In our previous study, we showed some features of IGC structure and molecular composition in oocyte nuclei of the scorpionfly, *Panorpa communis* [11]. Importantly, we found that along with splicing factor SC35, a marker protein of IGCs [12,13], *P. communis* oocyte IGCs contain two (unphosphorylated and hyperphosphorylated) forms of RNA polymerase II (Pol II) [11]. In the present study, we extend these data attempting to localize in the IGCs of *P. communis* oocytes additionally to Pol II some other components of Pol II holoenzyme and factors involved in activation of Pol II transcription. It is known that Pol II holoenzyme consists of core enzyme Pol II, general transcription factors, and the core Srb-mediator complex [14,15]. It should be noted that the participation of IGCs in nuclear distribution of the Pol II holoenzyme components remains to be more learned particularly in such highly specific cells as oocytes. Besides, the rare studies on this problem have been predominantly concerned the active state of the oocyte nucleus [16]. Therefore, it would be interesting to trace a behaviour of Pol II holoenzyme components in association with the IGCs in transcriptionally inert oocytes. It is the reason for choosing of *P. communis* diplotene oocytes as an experimental model, because they possess a fully inactivated nuclei at this stage of oogenesis when chromosome condensation and karyosphere formation occur [11,17,18]. Thus, using immunogold labeling/electron microscopy we have mapped inside the diplotene oocyte IGCs of *P. communis* the following component of Pol II holoenzyme: Pol II itself and basal transcription factors, TFIID and TFIIH. Additionally, we have defined the localization of transcriptional coactivators, CBP/p300, in oocyte IGCs of this species.

Material and methods

The specimens of the scorpionfly, *Panorpa communis* L. were collected in June in the village of Toksovo (Leningrad region, Russia).

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Folia Histochem Cytobiol. 2009:47(1): 123 (123-126)
doi: 10.2478/v10042-009-0006-2

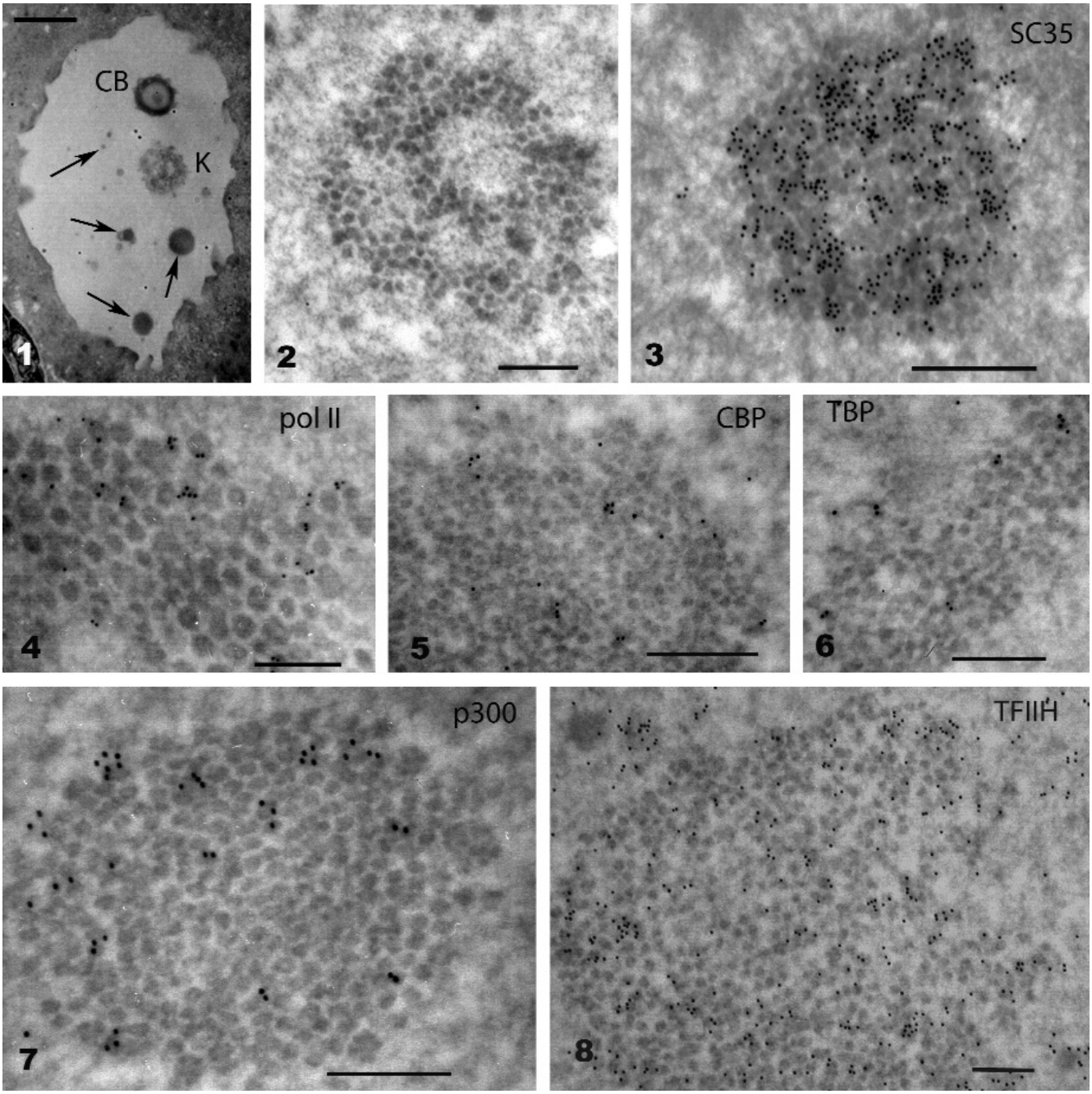

Fig. 1. A semithin section of *Panorpa communis* diplotene oocyte nucleus at the stage of late previtellogenesis. The largest nuclear body is the Cajal body (CB). Nuclear bodies (arrows) and a karyosphere (K) are seen in the nucleoplasm. Bar = 5 μm. **Fig. 2.** Interchromatin granule cluster of *P. communis* oocytes as viewed by routine electron microscopy. Bar = 0.5 μm. **Fig. 3-8.** Interchromatin granule clusters of *P. communis* oocytes after immunogold labeling with antibodies against non-snRNP splicing factor SC35 (Fig. 3), hyperphosphorylated C-terminal domain (CTD) of the Pol II (Fig. 4), C-terminal domain of transcription coactivator CBP (Fig.5), full length TATA-binding protein (TBP) (Fig.6), N-terminal domain of transcription coactivator p300 (Fig. 7), and basal transcription factor TFIIH (Fig.8). Bar = 0.5 μm.

Ovarioles were prepared for immunogold labeling electron microscopy as described [11]. Primary antibodies included the following monoclones: αSC35 against the non-snRNP splicing factor SC35 [12], V22 against the hyperphosphorylated C-terminal domain (CTD) of the Pol II, and the following rabbit polyclonal sera: N-17 against the component of the basal transcription factor TFIIH (Santa Cruz Biotechnology, Inc.), N-15 against the N-terminal domain of transcription coactivator p300 (Santa Cruz Biotechnology, Inc.), SI-1 against the full length TATA-binding protein (TBP) (Santa Cruz Biotechnology, Inc.), N-20 against the C-terminal domain of transcription coactivator CBP (Santa Cruz Biotechnology, Inc.).

Secondary antibodies were goat anti-mouse or goat anti-rabbit antibodies conjugated with colloidal gold particles of 10 nm (BBInternational).

Folia Histochem Cytobiol. 2009:47(1): 124 (123-126)
doi: 10.2478/v10042-009-0006-2

Results and discussion

The nuclear morphology of *P. communis* oocytes presented here corresponds to that described earlier [11]. The general appearance of diplotene oocyte nucleus at the stage of late previtellogenesis is shown in Fig. 1. The nucleus contains a complex of the karyosphere consisting of highly condensed chromosomes and numerous nuclear bodies (NBs); some of them are also distributed in nucleoplasm. In the previous study, the largest NB was identified as a (CB) [11]; other NBs composed of a granular material (Fig. 2) contain the marker component of IGCs – SC35 (Fig. 3) and, thus, may be reffered to as *P. communis* oocyte IGCs. The granules of *P. communis* oocyte IGCs range between 30-50 nm, i. e. they are larger than those in the canonical IGCs of mammalian somatic cells where they are 20-25 nm in diameter [19]. Immunogold labeling with anti-SC35 antibody shows that all *P. communis* oocyte IGCs significantly accumulate IGC marker protein SC35 (Fig. 3). The IGCs were also labeled with antibodies against the Pol II (Fig. 4), transcriptional coactivators CBP (Fig.5) and p300 (Fig. 7), and both basal transcription factors TBP (Fig. 6) and TFIIH (Fig. 8). It should be mentioned that the experiments with all these antibodies revealed the same pattern of labeling: the labels were predominantly associated with the granules of IGC. The revealed association of Pol II holoenzyme component (Pol II and basal transcription factors) with IGCs granules of inactive oocytes is significant. In transcriptionally active cells, these data could be explained in the frame of the suggestion that each granule, or "Pol II transcriptosome", contains Pol II holoenzyme prior the transfer of a transcriptosome to active chromatin as it was postulated for the granules of Xenopus oocyte CBs [16]. At the same time, Xenopus oocyte IGCs (B-snurposomes) do not contain Pol II [20]. However, *P. communis* oocyte IGCs are unusual IGCs because they also contain the CB marker protein coilin [11] and, thus, may share some features of both IGCs and CBs. It should be also noted that *P. communis* diplotene oocytes used in this study are transcriptionally inert [17] and, in contrast to the active cells, the IGC granules of *P. communis* oocytes could be the storage sites for the Pol II holoenzyme components disengaged from transcription. The same function of IGCs in inactive insect oocytes was discussed earlier [6,8]. Additionally, our data on tight association of transcriptional coactivators CBP/p300 with granules of IGCs in *P. communis* oocytes (Fig. 5, 7) suggest the CBP/p300 which is not a part of the holoenzyme, does connect with the transcriptosomes of IGCs in inactive oocyte nuclei. In this case, the presence of the CBP/p300 in IGCs is consistent with observations on the accumulation of CBP/p300 in IGCs of some cells when Pol II transcription was inhibited by drugs [21].

Acknowledgements: The authors are indebted to the following people providing us with antibodies: J.G. Gall (αSC35) and U. Scheer (V22). We also thank Dr. D. Bogolyubov for collecting the specimens and critical reading of the manuscript and Yu. I. Gukina for technical assistance. This work was supported by Russian Foundation for Basic Research (grant No. 06-04-48904, 09-04-00723) and by the grant program "Molecular and Cellular Biology".

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Submitted: 7 July, 2008
Accepted after reviews: 12 October, 2008

Folia Histochem Cytobiol. 2009:47(1): 126 (123-126)
doi: 10.2478/v10042-009-0006-2