

Interchromatin granule clusters of the scorpionfly oocytes contain poly(A)⁺ RNA, heterogeneous ribonucleoproteins A/B and mRNA export factor NXF1

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Abstract

IGCs (interchromatin granule clusters), or nuclear speckles, are one of the most universal subnuclear organelles of eukaryotic cells. We have used insect oocytes to study the possible association of poly(A)⁺ RNA and some factors involved in mRNA export with IGCs. Oogenesis of the mecopteran, *Panorpa communis*, used as a model object, is characterized by a strict cessation of oocyte genome transcription activity towards the end of oocyte growth. Our previous studies on *P. communis* oocyte nuclei have shown that oocyte IGC counterparts in this species are very unusual, both in morphology and molecular composition, compared with the typical IGCs of mammalian somatic cells traditionally used as a model system. We have now used microinjections of 2'-O-Me(U)₂₂ probes conjugated with the fluorochrome TAMRA to localize poly(A)⁺ RNA in IGCs. RNA export proteins were also detected by immunofluorescent/confocal and immunogold labelling electron microscopy. We found that poly(A)⁺ RNA, hnRNPs A/B and NXF1 mRNA export factors are located in IGCs regardless of the transcriptional status of the nucleus. Our data support the idea of IGCs as universal and conserved nuclear domains that serve not only as splicing factor reservoirs, but also take part in mRNA retention and export.

Keywords: heterogeneous nuclear ribonucleoprotein; interchromatin granule cluster; nuclear compartmentalization; oocyte nucleus; NXF1; poly(A)⁺ RNA

1. Introduction

IGCs (interchromatin granule clusters) are one of the most universal, abundant and essential organelles (or domains) of the nucleus (Gall, 2003; Gall et al., 2004). They are also known as nuclear (splicing) speckles, splicing factor compartments or SC35 domains and can be found in cells of evolutionarily diverse organisms, from plants (Lorković and Barta, 2004) and protists (Alverca et al., 2006) to mammals and humans [reviewed by Lamond and Spector (2003)].

The main function of IGCs in the nucleus has been seen as the assembly, modification, temporary storage and/or recycling of pre-mRNA splicing factors, including small nuclear (sn) RNAs and SR proteins (Misteli and Spector, 1998; Misteli, 2000). According to the 'classical' view, IGCs/speckles are reservoirs, or depots, of splicing factors that are recruited to the active genes in actively transcribing cells, whereas splicing itself does not occur in IGCs (Jiménez-García and Spector, 1993; Mattaj, 1994; Zhang et al., 1994; Huang and Spector, 1996; Misteli et al., 1997). The SR protein SC35, the essential non-snRNP splicing factor, is highly concentrated in IGCs; thus, this protein may be a diagnostic marker of IGCs/speckles (Fu and Maniatis, 1990; Spector et al., 1991; Spector, 2006).

However, some recent studies showed that IGCs are not simple stores of inert splicing factors, but are actively involved in

many other nuclear functions (Hall et al., 2006). IGCs might provide efficient coupling of transcription initiation, elongation and pre-mRNA processing to enhance the co-ordinated regulation of gene expression multistage events (Sacco-Bubulya and Spector, 2002; Hu et al., 2008). These domains are now considered as functional centres of local euchromatin neighbourhoods that group many active genes together (Shopland et al., 2003; Zhao et al., 2009). Some authors believe that (pre-)mRNA transcripts can be structurally constrained within IGCs (Shopland et al., 2002) and retained therein to acquire the ability to export (Johnson et al., 2000; Schmidt et al., 2009).

Some data indicate the presence of poly(A)⁺ RNA in IGCs (Visa et al., 1993; Molenaar et al., 2004; Ishihama et al., 2008). Furthermore, some poly(A)⁺ RNA located in IGCs is believed to represent mRNA or pre-mRNA (Shopland et al., 2002; Molenaar et al., 2004). Pre-mRNA accumulates in IGCs in an intron-dependent manner (Ishihama et al., 2008). After splicing, mRNA is exported to the cytoplasm by an active energy-requiring process (Tokunaga et al., 2006).

Nascent mRNA in the form of hn (heterogeneous nuclear) RNAs are associated with a set of proteins to form hnRNPs (hn ribonucleoproteins). Several hnRNP proteins shuttle continuously and rapidly between the nucleus and the cytoplasm and are associated with mRNA in both compartments. Others do not shuttle and are retained in the nucleus. Shuttling hnRNP proteins are the best candidates for mRNA export mediators (Piñol-Roma

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Abbreviations: CB, Cajal body; hn, heterogeneous nuclear ribonucleoprotein; hnRNP, heterogeneous nuclear ribonucleoprotein; ICG, interchromatin granules cluster; RNAP, RNA polymerase II.

and Dreyfuss, 1992, 1993; Mili et al., 2001). For example, hnRNP A1 is bound to poly(A)⁺ RNA and exported from the nucleus together with the mRNA (Piñol-Roma and Dreyfuss, 1992). This is the best characterized hnRNP protein that belongs to the A/B subfamily (hnRNPs A/B). hnRNPs A/B are among the most abundant hnRNP proteins, contributing to many nuclear functions including mRNA export [for a review, see the work of He and Smith (2009)]. Significantly, vertebrate hnRNPs A/B contain highly conserved sequences that allow immunological detection of the related proteins by the same antibody in divergent organisms, including insects (Matunis et al., 1992; Visa et al., 1996).

The factors required for poly(A)⁺ mRNA export are also conserved between divergent organisms, from yeast to humans, and include the export receptor NXF1 (Grüter et al., 1998). The orthologue of vertebrate NXF1 has also been characterized in the insect *Drosophila melanogaster* and is essential for mRNA export (Wilkie et al., 2001).

Typical IGCs represent irregularly shaped nuclear bodies that consist of 20- to 25-nm granules embedded in a fine fibrillar matrix (Monneron and Bernhard, 1969; Puvion and Moyne, 1981). Comparative ultrastructural studies showed that in oocytes of different insects, fine structure and molecular composition of IGCs may differ from typical IGCs of mammalian somatic cells or amphibian oocytes traditionally used as models to investigate IGC structure and functions [reviewed by Bogolyubov and Parfenov (2008), Bogolyubov et al. (2009)]. For instance, the IGCs of the scorpionfly (*Panorpa communis*) oocytes contain granules about twice the size of mammalian somatic-cell IGCs (30–50 nm) and characteristically include prominent zones of segregated fine-fibrillar material of unknown nature distinguished by the packing density of the fibrils (Batalova et al., 2000, 2005a, 2005b).

P. communis oocyte IGCs were also shown to contain both non-phosphorylated and hyperphosphorylated forms of RNAP (RNA polymerase II) and some other essential factors associated with RNAP II transcription (Batalova et al., 2005a; Batalova and Parfenov, 2009). This differs from some results on IGC's association with RNAP II in other cells. Some authors have shown that either IGCs are devoid of RNAP II (Doyle et al., 2002; Guillot et al., 2004), or they contain inactive phosphorylated RNAP II (Xie et al., 2006).

Taken together, a number of noticeable characteristics of the structure and composition of *P. communis* oocyte IGCs can pose the question whether so unusual IGCs play a functional role similar to that mentioned above for mammalian somatic-cell IGCs. We believe that this specific issue could be addressed by studying the association of poly(A)⁺ RNA and some factors of its export using *P. communis* oocyte IGCs. To this end, we used microinjections of fluorescently tagged 2'-O-Me(U)₂₂ oligonucleotide probes to localize poly(A)⁺ tails of RNA in transcriptionally active and inert nuclei of *P. communis* oocytes.

It should be mentioned that we chose *P. communis* oocytes as a model system because this system offers stages of development that possess a spectrum of transcriptional activity ranging from active to inert. In *P. communis* oogenesis, the transcriptional inactivation of oocyte genome occurs towards the end of oocyte growth. Simultaneously, oocyte chromosomes condense to form the so-called karyosphere or karyosome (Gruzova and Parfenov, 1993). Fully developed karyospheres of *P. communis* oocytes do

not incorporate bromouridine (Bogolyubov, 2007). IGCs/speckles in the nuclei were revealed immunocytochemically by counterstaining with an antibody against SC35 protein. We also localized mRNA export factor NXF1 and hnRNP A/B proteins in oocyte IGCs using immunofluorescent/confocal and immunogold labeling/electron microscopy. The data obtained here are in a good agreement with the hypothesis that IGCs are involved in mRNA metabolism.

2. Materials and methods

2.1. Animals and tissue preparation

Specimens of *P. communis* L. (Mecoptera: Panorpidae) were collected in late June in the village of Toksovo near the lake Vääräjärvi (Leningrad Region, Russia). Ovaries and separate ovarioles were isolated in a solution for insects containing 0.75% NaCl, 0.035% KCl and 0.0021% CaCl₂. Ovarioles were lightly squashed on a coverslip with a glass slide (Hulsebos et al., 1984; Liu et al., 2006). The squashes were frozen in liquid nitrogen, fixed in 2% formaldehyde freshly prepared from paraformaldehyde (Ted Pella), in 96% ethanol for 45 min, and rinsed in 70% ethanol and finally in PBS.

2.2. Antibodies

Primary antibodies for immunostaining included monoclonal antibody against non-snRNP splicing factor SC35 (Fu and Maniatis, 1990) and the following rabbit polyclonal antibodies: H-120 against amino acids 1–120 of TAP/NXF1 and H-200 against amino acids 1–200 of hnRNPs A/B (both from Santa Cruz Biotechnology). Secondary antibodies were FITC of Alexa 594-conjugated goat anti-mouse IgG and goat anti-rabbit IgG (Molecular Probes).

2.3. Microinjections

Microinjections of 2'-O-Me(U)₂₂ probes conjugated with TAMRA (Molecular Probes) were performed to localize poly(A)⁺ RNA. The probes were synthesized by Sintol Company. Microinjections into the oocytes were performed using an Eppendorf 5242 microinjector and a Leica DM IRB microscope equipped with a Narishige micromanipulator. After microinjection, the ovarioles were kept in a moist chamber for 1–2 h, squashed and fixed as described above, and counterstained with anti-SC35 antibody to localize nuclear speckles/IGCs. Control preparations were additionally incubated with 20 µg/ml RNase A (Sigma) for 1.5 h at 37°C.

2.4. Immunofluorescent/confocal microscopy

Preparations were incubated in 10% fetal calf serum (Gibco) in PBS for 10 min to prevent non-specific antibody binding. The incubation in the primary antibody solution was carried out overnight in a moist chamber at 4°C. After rinsing in PBS, the preparations were incubated with secondary antibodies for 1.5 h

at room temperature. After rinsing in PBS, the preparations were stained with a DNA-specific dye, To-Pro-3 (Molecular Probes) for 1 min, rinsed in PBS and mounted in Vectashield medium (Vector Laboratories). The samples were examined with a Leica TCS SL confocal laser scanning microscope equipped with argon (488 nm) and helium–neon (543 and 633 nm) lasers. Confocal images were taken with a $\times 63$ (NA 1.32) objective. Merged images were obtained by using Leica Confocal Software or ImageJ 1.32a. Contrast and relative intensities of images were adjusted with Adobe Photoshop.

2.5. Immunogold labelling electron microscopy

Single ovarioles were prefixed for 1.5 h in 4% formaldehyde freshly prepared from paraformaldehyde and 0.5% glutaraldehyde in PBS, and fixed overnight in 2% formaldehyde at 4°C. After rinsing in PBS containing 0.05 M NH_4Cl for 10 min and subsequent dehydration in an ethanol series, ovarioles were embedded in LR White resin of medium grade (Sigma). Ultrathin sections were prepared with a Reichert–Jung ultracut microtome and incubated for 10 min in a blocking buffer containing 0.5% fish gelatin (Sigma) and 0.02% Tween-20 in PBS, pH 7.4. After blocking, the sections were incubated in primary antibody solution overnight in a moist chamber at 4°C. Following rinsing in PBS, the sections were incubated with secondary antibodies conjugated to 10 or 15 nm colloidal gold particles (BBInternational). As a control, additional sections were incubated only in secondary antibodies. The sections were contrasted with uranyl acetate (Electron Microscopy Sciences) and examined in a Jeol 7A or Jeol 100C electron microscope at 80 kV.

3. Results

Two opposite stages of *P. communis* oogenesis characterized by transcriptionally active or inert oocyte nuclei and have been distinguished previously using bromo-UTP microinjections into the oocytes (Bogolyubov, 2007) were used. The chromosomes of early previtellogenic oocytes are long, loosened and occupy most of the nucleus. Inactivation of oocyte chromosomes during their growth is accompanied by their condensation. The condensed chromosomes occupy a very limited area of the large nucleus to form the so-called karyosphere or karyosome (Batalova et al., 2005b). The condensed chromosomes in the karyosphere are embedded in the fibrogranular material similar to that of free nucleoplasmic nuclear bodies. The IGC counterparts have previously been identified in *P. communis* oocytes as morphologically complex nuclear bodies enriched in pre-mRNA splicing factors, RNAP II (Batalova et al., 2005a, 2005b), transcription co-activators CBP/p300 and the basal transcription factor TFIIF (Batalova and Parfenov, 2009).

Our microinjection experiments with TAMRA-conjugated 2'-O-Me(U)₂₂ oligonucleotide probes that reveal poly(A)⁺ RNA tails with high efficiency (Majlessi et al., 1998; Molenaar et al., 2001) showed poly(A)⁺ RNA in many SC35-positive domains (IGCs/speckles) of *P. communis* oocyte nuclei (Figures 1, 2). In the transcriptionally

active nucleus of early diplotene oocytes, the main portion of SC35 domains (=speckles/IGCs) contained poly(A)⁺ RNA (Figure 1); however, the presence of poly(A)⁺ RNA in several of the smallest domains was not so evident (Figure 1, inset). Poly(A)⁺ RNA localized predominantly to the central part of SC35 domains. Thin peripheral zones of these domains appeared devoid of poly(A)⁺ RNA. In the transcriptionally silent nucleus of vitellogenic oocytes, strong co-localization of poly(A)⁺ RNA and SC35 protein in all SC35 domains was observed (Figure 2). Both SC35-positive material that is associated with the condensed chromosomes forming the karyosphere and free SC35 domains scattered in the rest of the nucleoplasm, contained poly(A)⁺ RNA. In preparations treated with RNase, only the SC35-positive signal was registered (not shown).

Double immunofluorescent staining with antibodies against SC35 protein and hnRNPs A/B showed strong co-localization of these proteins in speckles/IGCs in both transcriptionally active (Figure 3) and silent (Figure 4) oocytes. At the ultrastructural level, *P. communis* oocyte IGCs were also labelled with both anti-SC35 and anti-hnRNPs A/B antibodies (Figures 7A, 7B).

Confocal microscopy also demonstrated the co-localization of mRNA export factor NXF1 and SC35 protein in speckles/IGCs both in transcriptionally active and inert oocyte nuclei (Figures 5, 6). Immunogold labelling electron microscopy confirmed the presence of NXF1 in IGCs (Figure 7C).

4. Discussion

We have analysed the distribution of poly(A)⁺ RNA and some molecular compounds linked to mRNA export in transcriptionally

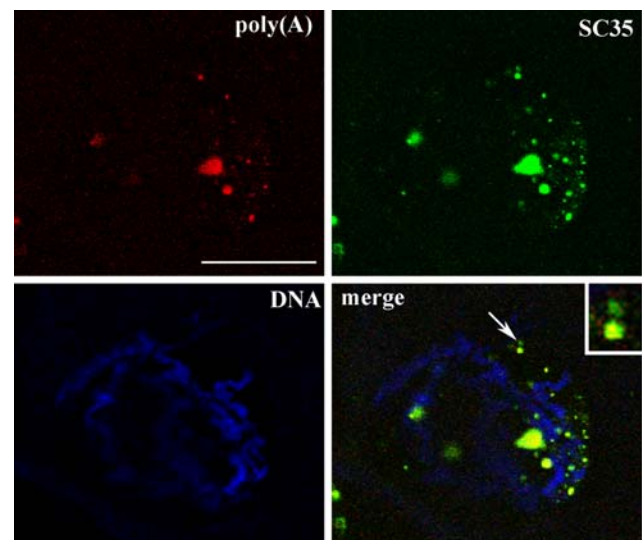


Figure 1 Nuclear speckles/IGCs in transcriptionally active nucleus of early previtellogenic (early diplotene stage) oocyte of *P. communis* after microinjection of 2'-O-Me(U)₂₂ oligonucleotide probe to reveal poly(A)⁺ RNA (red) and immunostaining with anti-SC35 antibody (green)

Poly(A)⁺ is seen in most of the SC35 domains. Some small domains, however, do not contain a significant amount of poly(A)⁺ RNA (arrow and insert in the merged image at higher magnification), DNA was stained with To-Pro-3 (blue). Note in inset the location of poly(A)⁺ RNA in the central zone of large speckle (yellow dots). Scale bar=20 μm .

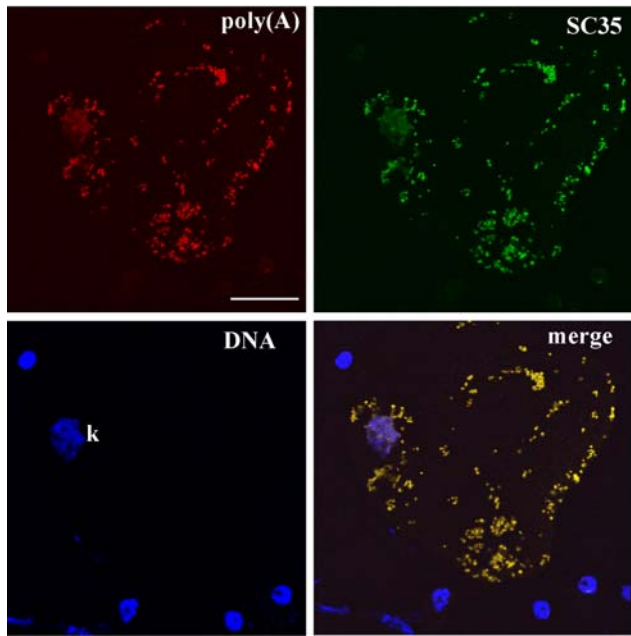


Figure 2 Nuclear speckles/IGCs in transcriptionally silent nucleus of early vitellogenic (late diplotene stage) oocyte of *P. communis* after microinjection of 2'-O-Me(U)₂₂ oligonucleotide probe to reveal poly(A)⁺ RNA (red) and immunostaining with anti-SC35 antibody (green)

Poly(A)⁺ RNA is found in all SC35 domains. DNA was stained with To-Pro-3 (blue). Oocyte chromosomes at this stage are condensed and united into the karyosphere (k). Scale bar=20 μm.

active and inert nuclei of insect oocytes. Inactivation of the oocyte genome seems to be a rule for organisms with 'nutritional' oogenesis when growing oocytes are accompanied by polyploid and highly active nurse cells that provide an oocyte with RNA (Berry, 1985). Correspondingly, oocyte chromosomes of such animals condense to form a karyosphere at a defined stage of oocyte growth (Gruzova and Parfenov, 1993; Bogolyubov and Parfenov, 2008). Cessation of transcriptional activity of oocyte nuclei during *P. communis* oogenesis were previously documented after bromo-UTP microinjection experiments (Bogolyubov, 2007) and immunogold labelling electron microscopy with a set of antibodies against certain essential factors of gene expression (Batalova et al., 2005a, 2005b).

A key point that for a long time has needed to be established is whether SC35 domains (speckles/IGCs) contain any mRNA in transit to the cytoplasm (Lamond and Spector, 2003). We found that poly(A)⁺ RNA is associated with IGCs in both transcriptionally active and silent oocyte nuclei. We also found that hnRNPs A/B and NXF1 are localized to IGCs regardless of the transcriptional status of the nucleus. Our data are consistent with the current notion of a direct role of IGCs in mRNA metabolism (Hall et al., 2006).

The presence of poly(A)⁺ RNA in IGCs has been documented for mammalian somatic cells (Carter et al., 1991, 1993; Visa et al., 1993; Huang et al., 1994) and early embryos (Bogolyubova et al., 2009). However, the nature of this RNA remains questionable. It is generally agreed that transcription does not occur in most IGCs (Cmarko et al., 1999), although Wei et al. (1999) take a different viewpoint. Whatever

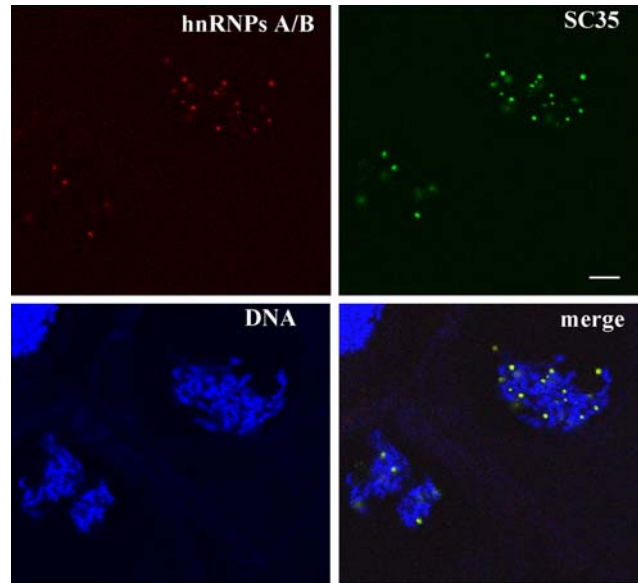


Figure 3 An active stage oocyte nucleus of *P. communis* (early diplotene/early previtellogenesis) after immunostaining with antibodies against hnRNPs A/B (red) and SC35 protein (green), DNA was stained with To-Pro-3 (blue)

hnRNPs A/B are revealed in all SC35 domains. At this stage, chromosomes occupy the essential part of the nucleus. Scale bar=8 μm.

the case, some authors argue in favour of the poly(A)⁺ RNA in many IGCs being comprised of, at least in part, mRNA or pre-mRNA. First, about 75% of nuclear poly(A)⁺ RNAs pass through IGCs in

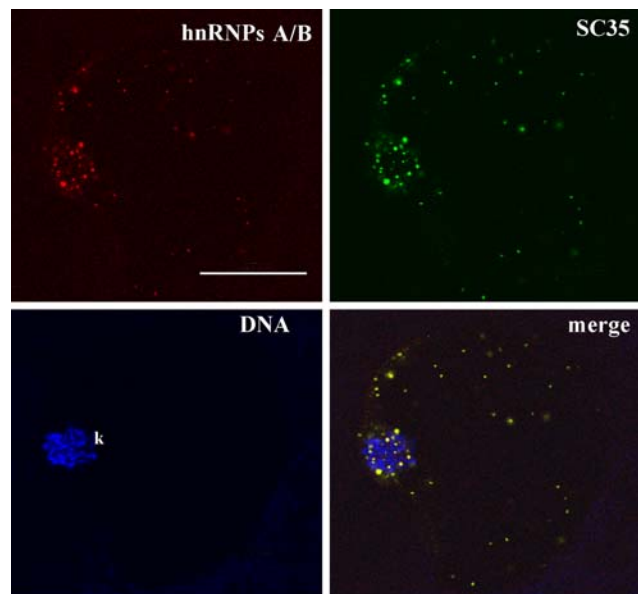


Figure 4 An inactive stage oocyte nucleus of *P. communis* (late diplotene/late vitellogenesis) after immunostaining with antibodies against hnRNPs A/B (red) and SC35 protein (green), DNA was stained with To-Pro-3 (blue)

Oocyte chromosomes at this stage are united into the compact karyosphere (k). Note that hnRNPs A/B are seen in SC35 domains, both associated with the karyosphere and scattered freely in the nucleoplasm. Scale bar=20 μm.

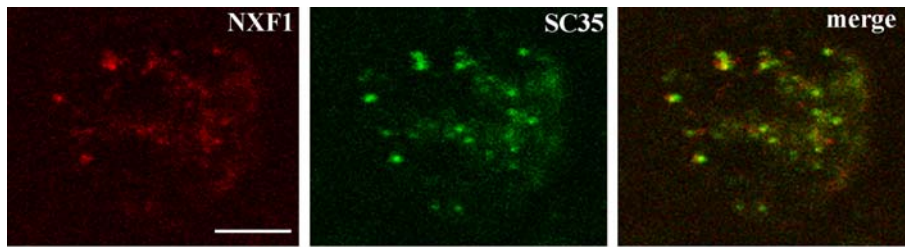


Figure 5 An active stage oocyte nucleus of *P. communis* (late pachytene/early diplotene) after immunostaining with antibodies against NXF1 (red) and SC35 proteins (green)
Scale bar=4 μm .

mammalian somatic cells (Molenaar et al., 2004). Secondly, intron-containing pre-mRNA microinjected into HeLa cells is distributed to IGCs and exported to the cytoplasm after splicing (Tokunaga et al., 2006). Thirdly, the assembly of pre-spliceosomal complexes on splicing-competent pre-mRNA can be undertaken in IGCs (Melčák et al., 2001). Finally, the kinetic analysis of microinjected pre-mRNA has shown that intron-containing pre-mRNA may repeatedly join and leave IGCs until the introns are removed (Ishihama et al., 2008).

It is not absolutely clear whether poly(A)⁺ RNA is a resident component of IGCs. Some authors believe that mRNP movement is governed by simple diffusion, i.e. is not directional, and that it is rarely hindered by stable interactions with nuclear substructures (Shav-Tal et al., 2004). Other authors, however, found evidence that some mRNA transcripts within IGCs are not free to diffuse out of them and can be structurally constrained within these domains (Shopland et al., 2002).

Dynamic association of poly(A)⁺ RNA with IGCs also continues to occur in cells treated with transcription inhibitors (Shopland et al., 2002; Molenaar et al., 2004; Politz et al., 2006). Here, we similarly

found that poly(A)⁺ RNA is retained in IGCs of transcriptionally silent insect oocytes under physiological conditions when a karyosphere develops in the nucleus of growing oocytes.

Some factors directly involved in mRNA export were also demonstrable in IGCs of mammalian somatic cells (Zhou et al., 2000; Degot et al., 2004; Schmidt et al., 2006, 2009). These findings favour the supposition that mRNAs in IGCs may acquire their ability to export (Johnson et al., 2000; Schmidt et al., 2009). It is known that mRNA export is mediated by a special group of proteins (Erkmann and Kutay, 2004). The essential export factor for >75% of analysed mRNAs is NXF1 (also known as TAP in human cells) (Grüter et al., 1998). There is no data on the localization of NXF1 in the 3D structure of the nucleus. However, close association with IGCs has been reported for another protein, Aly, that links pre-mRNA splicing to mRNA export (Zhou et al., 2000).

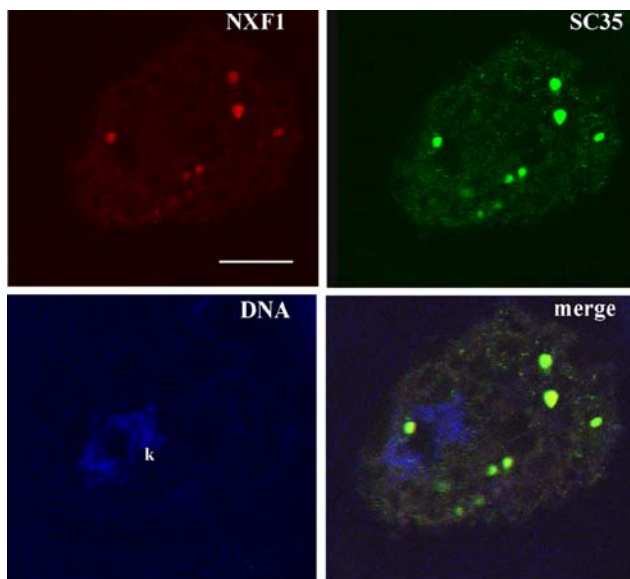


Figure 6 An inactive stage oocyte nucleus of *P. communis* (diplotene/late previtellogenesis) after immunostaining with antibodies against NXF1 (red) and SC35 proteins (green)
Oocyte chromosomes are united into the perfectly developed karyosphere (k, blue) at this stage. Scale bar=8 μm .

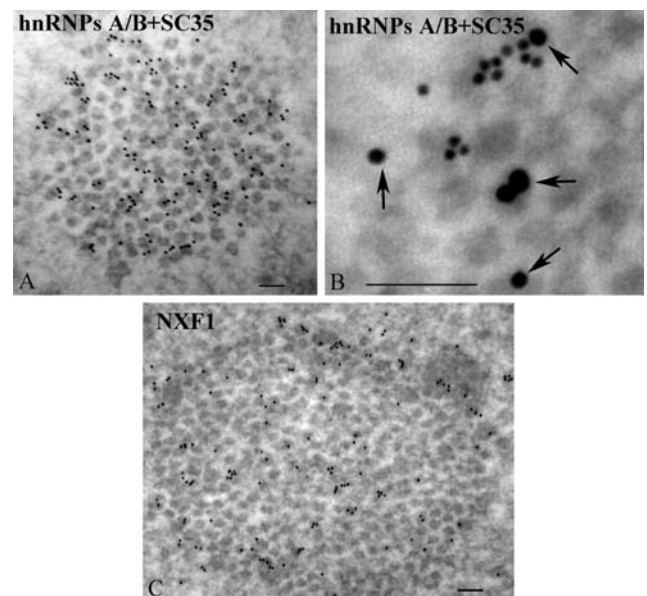


Figure 7 Immunoelectron revealing of SC35, hnRNPs A/B and NXF1 in IGCs of *P. communis* oocytes

(A) An IGC after double labelling with anti-SC35 (10-nm gold particles) and anti-hnRNPs A/B (15-nm gold particles) antibodies. (B) A part of an IGC after double labelling with the same antibodies as in (A) at higher magnification to show the co-localization of SC35 and hnRNPs A/B within the IGC; arrows indicate 15-nm gold particles that correspond to hnRNPs A/B. (C) An IGC after labelling with anti-NXF1 antibody. Scale bars=0.1 μm .

The NXF family of proteins is evolutionarily conserved. The orthologs of human TAP/NXF1 and yeast Mex67p have been described in a variety of organisms (Erkman and Kutay, 2004). There is a valid suggestion that NXF1 is a general mRNA export factor in all eukaryotes, including insects (Herold et al., 2001; Wilkie et al., 2001).

We have found that IGCs observed in transcriptionally active and silent oocyte nuclei of an insect contain hnRNPs A/B. This group of proteins was recently shown to localize in IGCs of two-cell mouse embryos in an RNA-dependent manner (Bogolyubova et al., 2009). It is well-known that in eukaryotic cells, pre-mRNA transcripts are associated with a set of specific proteins to form hnRNP complexes. These complexes are the substrates for post-transcriptional modifications of pre-mRNA (Dreyfuss et al., 1993). Pre-mRNA-binding proteins, collectively called hnRNP proteins, include about 20 major polypeptides that can be placed into a few general subfamilies of the proteins. The hnRNPs A/B comprising A1, A2/B1, A3 and A0 proteins are among the most abundant hnRNP core proteins that are involved in many nuclear functions, including chromosome maintenance, DNA replication and repair, transcription, splicing and mRNA export (He and Smith, 2009). Significantly, hnRNPs A/B share a high level of sequence identity (He and Smith, 2009) and show high conservation in divergent organisms, both vertebrates and invertebrates including insects (Matunis et al., 1992; Visa et al., 1996).

Several hnRNP proteins, including some hnRNPs A/B, shuttle continuously and rapidly between the nucleus and the cytoplasm and are associated with mRNA in both compartments. Thus, these proteins may serve as mRNA export mediators (Piñol-Roma and Dreyfuss, 1992, 1993). Of these, the best studied example is A1. Microinjection experiments with *X. laevis* oocytes have directly implicated A1 in mRNA export due to the M9 domain that mediates both nuclear export and import (Izaurralde et al., 1997). A1 binds poly(A)⁺ RNA in both the nucleus and cytoplasm (Piñol-Roma and Dreyfuss, 1992) and accompanies mRNA through the nuclear pores to polysomes as observed by immunoelectron microscopy (Visa et al., 1996).

It is significant that the distribution of A1 in the nucleus differs markedly from that of other hnRNPs A/B. Moreover, A2/B1 does not associate with IGCs as well as with some other prominent nuclear domains, including CBs (Cajal bodies) and their related structures, Gems (Friend et al., 2008). Thus, we could expect that a significant portions of hnRNPs A/B revealed in *P. communis* oocyte IGCs comprises A1.

The revealed heterogeneity of the IGC population in transcriptionally active nuclei of *P. communis* oocytes may be explained by the high rate of macromolecular exchange between IGCs and the rest of the nucleoplasm. However, real heterogeneity of the IGC population was recently established for plant cells in respect of SR proteins (Lorković et al., 2008). The authors showed that different SR proteins localize to different IGCs. It cannot be excluded that similar heterogeneity might characterize IGCs of other organisms and apply to many other IGC compounds. Our observations that poly(A)⁺ RNA co-localizes with SC35 protein in many, but not all, IGCs of active stage oocytes are in agreement with this notion. On the contrary, strong co-localization of SC35 protein with poly(A)⁺

RNA as well as with hnRNPs A/B or NXF1 is evident for all IGCs of transcriptionally inert oocyte nuclei.

The function of extrachromosomal domains in transcriptionally inert oocyte nuclei of different insects is not clear. These domains, including IGCs, are enriched in RNAP II and pre-mRNA splicing factors (Bogolyubov et al., 2000; Jaglarz, 2001; Bogolyubov and Parfenov, 2001, 2004; Batalova et al., 2005a, 2005b). The domains may represent storage/recycling sites for the compounds disengaged from RNA transcription/processing cycles (Bogolyubov and Parfenov, 2008). Therefore, the finding of poly(A)⁺ RNA and mRNA export factors in IGCs of transcriptionally inert *P. communis* oocytes is not unexpected. Further investigations are needed, however, to understand better the composition and function of oocyte extrachromosomal domains.

5. Conclusion

In spite of the undoubted recent progress in deciphering of the functions of nuclear subdomains including IGCs, CBs and some others, this problem is far from being solved. The use of traditional model systems like mammalian tissue-culture somatic cells or even the oocytes of *Xenopus* or *Drosophila* cannot entirely overlap the significant morphological and biochemical variety and complexity of nuclear bodies observed in the oocytes of invertebrates, mainly insects (Bogolyubov et al., 2009).

Our present study continued previous investigations on the miscellaneous nuclear structures of *Panorpa* oocytes (Gruzova, 1962; Ramamurty, 1963; Batalova and Tsvetkov, 1998; Batalova et al., 2000, 2005a, 2005b; Batalova and Parfenov, 2003, 2009). We found that IGC counterparts of *P. communis* oocytes contain some general factors that characterize IGCs of different cells and may indicate common functions of IGCs in cells of different origin and evolutionarily divergent organisms. We also found in this context that there is a heterogeneity of IGC population in transcriptionally active insect oocytes: a small portion of IGCs did not contain significant amount of poly(A)⁺ RNA. Our data also suggest that the presence of given factors in IGCs does not depend on the transcriptional state of the oocyte nucleus. We believe that further comparative studies on different and evolutionarily divergent organisms are clearly needed to extend our knowledge of the structure and functions of extrachromosomal subnuclear domains.

Author contribution

Florina Batalova performed immunofluorescence/confocal microscopy and immunogold microscopy. Dmitry Bogolyubov was involved in collection of specimens, microinjections, writing the text and preparing the manuscript. Vladimir Parfenov was involved with scientific supervision, project design and discussion of the manuscript.

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