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Nuclear bodies from cricket and mealworm oocytes contain splicing factors of pre-mRNA

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Gryllidae, *Acheta domesticus*, Tenebrionidae, *Tenebrio molitor*, oocytes, nuclear bodies, spheres, splicing factors

Abstract. A comparison of nuclear structures of the germinal vesicles from an insect with panoistic ovarioles (cricket *Acheta domesticus*) with that of an insect with meroistic ovarioles (mealworm *Tenebrio molitor*) was carried out. Immunocytochemistry revealed that spheres from cricket oocyte nuclei contained small nuclear RNP (snRNP) and fibrillarin – protein which may be involved in pre-rRNA processing. Additionally, coilin-related protein was revealed in cricket spheres. In the mealworm germinal vesicles, two types of nuclear bodies containing snRNP were identified. Only one of these types of nuclear bodies contained SC35 splicing factor. In situ, hybridization identified snRNAs U1, U2 and U6 in germinal vesicles of the mealworm and in spheres and karyoplasm of the cricket. After a prolonged incubation of ovaries in medium containing ^{3}H-uridine, the germinal vesicles were labeled; spheres of the cricket and nuclear bodies of the mealworm remained unlabeled. These results indicate that homology exists between spheres and nuclear bodies of insect germinal vesicles and that these are homologous to the coiled bodies of somatic cell nuclei and spheres of amphibian oocyte nuclei.

INTRODUCTION

Since the beginning of the century, numerous types of "nucleoli" have been described in nuclei of both invertebrates and vertebrates (Wilson, 1925). Some of these structures were not be regarded as true nucleoli because they did not contain rDNA (for review, see Gall, 1992). Bier et al. (1967) were first to study these structures in insect oocytes and called them "Binnenkörper". Using the method of autoradiography, they established that RNA was not synthesized in Binnenkörper, but accumulated there. Similar nuclear structures of germ cells were described by many authors under various terms. For example, nuclear organelles resembling Binnenkörper were described in some insects as "karyosphere nucleoli" or later as "nucleolus-like bodies" by Gruzova (1975, 1988). The similar structure in cricket oocytes named "Binnenkörper" (Bier et al., 1967) was later described as a "secondary nucleolus" (Allen & Cave, 1969; Javorska & Lima-de-Faria, 1973). Manchino et al. (1969) noted the resemblance between "secondary nucleoli" of *Acheta domesticus* and spheres from amphibian oocyte nuclei described by Gall (1954). The term "sphere organelles", or just "spheres", has also been used by Gall & Callan (1989) to designate "Binnenkörper" of crickets that were shown, through immunocytochemical techniques, to contain small nuclear RNPs (snRNP). Two main types of nuclear bodies (NB) were recently reported for oocytes of the mealworm *Tenebrio molitor* (Alexandrova, 1993; Alexandrova et al., 1995). NBs are often joined into multicomponent complexes. In *T. molitor*

* Deceased.

oocytes, part of the NB exists in close relationship to the karyosphere formed by meiotic bivalents (for review, see Gruzova & Parfenov, 1993). These NBs also contain snRNP (Alexandrova, 1993; Alexandrova et al., 1995).

Nuclear organelles containing snRNPs and other factors of pre-mRNA splicing and pre-rRNA processing have been described extensively. These organelles are coiled bodies in somatic cell nuclei (Fakan et al., 1984; Raška et al., 1991; Carmo-Fonseca et al., 1991, 1992, 1993; Frey & Matera, 1995), prenucleolar bodies in nuclei formed in vitro after adding DNA in *Xenopus laevis* egg extracts (Wu et al., 1993; Bauer et al., 1994), and spheres, or sphere organelles, in amphibian oocyte nuclei (Wu et al., 1991; Gall, 1992). Gall et al. (1995) suggested that coiled bodies, prenucleolar bodies and spheres are homologous structures.

An examination of similar nuclear organelles from oocytes of insects with different types of ovarioles would seem to be of special interest. Cricket ovarioles belong to panoistic type of ovary. They lack trophocytes and all oocyte RNA is synthesized by the germinal vesicle (Cave, 1973). Ovarioles of beetles, including *T. molitor*, are meroistic. The specialized trophocytes that occupy a terminal part of the germarium are connected with growing oocytes by trophic cords (Ullmann, 1973). In meroistic ovarioles nearly all RNA is delivered to oocytes from trophocytes (Capco & Jeffery, 1978, 1979).

The aim of this study is to elucidate whether NB from *A. domesticus* (sphere) and NB from *T. molitor* are homologous to each other and to the organelles mentioned above. In order to answer this question further immunocytochemical analysis was undertaken. Antibodies not only against snRNPs, but also against other factors of pre-mRNA splicing and pre-rRNA processing and against the protein p80-coilin were used. The protein p80-coilin is considered a marker for coiled bodies of somatic cells. In situ hybridization of U1, U2, and U6 snRNA antisense probes was carried out on squashed preparations and on histological sections to determine the localization of snRNAs in insect oocyte nuclei.

MATERIAL AND METHODS

Oocyte nuclei of the cricket *Acheta domesticus* L. (Ensifera: Gryllidae) and the mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) were studied. Insect ovaries were isolated in OR2 medium (Wallace et al., 1973). Squashes of cricket ovarioles and of isolated nuclei of mealworm oocytes were prepared according to Hulsebos et al. (1984). Nuclei were isolated and, if necessary, the nuclear content was dispersed according to Gall et al. (1981).

For the indirect immunocytochemical study, the squashes of ovarioles and spreads of nuclei were stained as described by Wu et al. (1991). Antibodies employed are presented in Table 1.

TABLE 1. Antibodies used for immunostaining.

Antibodies	Revealed antigens	References
mAb Y12	Sm-epitope of snRNP	Lerner et al., 1981
mAb K121	Trimethylguanosine cap of snRNA	Krainer, 1988
mAb αSC35	SR-protein, non-snRNA splicing factor of pre-mRNA	Fu & Maniatis, 1990
mAb 17C12	Fibrillarin	Ochs et al., 1985
Rabbit polyclonal serum R288	Carboxy-terminal fragment (14 kDa) of human p80-coilin	Andrade et al., 1993

Before ultrastructural immunocytochemistry, ovarioles were prefixed for 2 h in a solution containing 4% paraformaldehyde and 0.5% glutaraldehyde and then fixed overnight in 2% paraformaldehyde at 4°C. After rinsing in 1 × PBS containing 0.5 M NH_4Cl and undergoing dehydration, ovarioles were embedded in LR White resin (Polyscience, Warrington). Ultrathin sections were incubated, first in antibody solutions and then with secondary antibodies conjugated with colloidal gold particles (10 nm). For a control group, additional sections were incubated only in secondary antibodies.

To identify snRNA and rDNA in oocyte nuclei, the RNA-RNA and RNA-DNA in situ hybridization was carried out (Wu et al., 1991) on squashes and on paraffin sections of ovarioles. For synthesis of sense and antisense riboprobes, we used fragments of snRNA genes U1, U2, U6 and rDNA of *Xenopus laevis*, which were cloned in vector Bluescript. For synthesis of rRNA, we used EcoRI fragment of plasmid pXlr101B (Bakken et al., 1982); in the case of snRNA U1 and U2, we used fragments of *Drosophila melanogaster* genes (Mount & Steitz, 1981; Alonso et al., 1984); and in the case of snRNA U6, we used fragments of mouse U6 snRNA gene (Black & Pinto, 1989). To label riboprobes, we used in vitro transcription reaction with ^{3}H-UTP (148×10^{10} Bq/mM). Before hybridization, squashes or deparaffined sections were dehydrated with 100% ethanol and acetone and dried at room temperature. The hybridization solution consisted of 40% formamide, 4× SSC (SSC is 0.15 M NaCl, 0.015 M Na citrate, pH 7.0), 0.1 M Na_3PO_4, 300 μg/ml *Escherichia coli* DNA, 300 μg/ml *E. coli* tRNA and a ^{3}H-labeled probe. The specific activity of the probes was ~ 10^8 dpm/μg. Hybridization was carried out for 12–16 h at 42°C. Preparations were rinsed in 0.1× SSC at 60°C for 1 h to remove unhybridized probes. NTB-2 liquid emulsion (Eastman Kodak Co, Rochester, NY) was used for autoradiography. Preparations covered by emulsion were exposured for 24 h to 2 weeks. To study incorporation of ^{3}H-uridine into oocyte nuclei, gonads were incubated in OR2 medium with ^{3}H-uridine (0.8 Mbq/ml) for 1–24 h.

List of abbreviations used in figures: FC – follicular cell; GR – granules; K – karyosphere; N – nucleolus; NB – nuclear body; NB I – nuclear body of 1st type; NB II – nuclear body of 2nd type; OC – oocyte; ON – oocyte nucleus; PFC – prefollicular cell; S – sphere.

RESULTS

Nuclear organelles in *Acheta domesticus* oocytes

Indirect immunofluorescent staining of squashes of cricket ovarioles with monoclonal antibodies (mAbs) Y12, against Sm-epitope of snRNPs, and K121, against trimethylguanosine cap of snRNA, revealed spherical nuclear bodies in the nuclei of young previtellogenic oocytes (Figs 1A–G), which contained snRNPs. At this stage, the most prominent nuclear organelle is the nucleolus (Figs 1A, C). During oocyte growth this single nucleolus breaks down into thousands of small extrachromosomal nucleoli (Fig. 1E). Simultaneously, spheres increase in size (up to 15 μm) and their number decreases, perhaps as a result of their fusion. Large oocytes usually contain 1–2 spheres. Their structure, as a rule, is homogeneous (Figs 2A, C), but sometimes spheres contain vacuoles and granules (Fig. 2E).

Staining of cricket ovariole squashes with mAb 17C12 against fibrillarin, which is a protein of nucleolus fibrillar zone (Ochs et al., 1985), revealed that spheres, as well as nucleoli, are brightly stained (Figs 1E–F); i.e. spheres contain fibrillarin.

The most exciting results were obtained after treatment of cricket oocyte nuclei with polyclonal serum R288 against the protein p80-coilin. These antibodies are the only markers for the coiled bodies in somatic cell nuclei. Spheres were shown to interact with these antibodies specifically (Figs 2A–B). Control preparations treated with preimmune serum from the same source rabbit were not stained (Figure not shown). Thus, spheres from *A. domesticus* oocytes contain coilin or coilin-like protein. The staining of spheres with all of the antibodies mentioned above did not differ depending on the internal structure of spheres and was homogeneous.

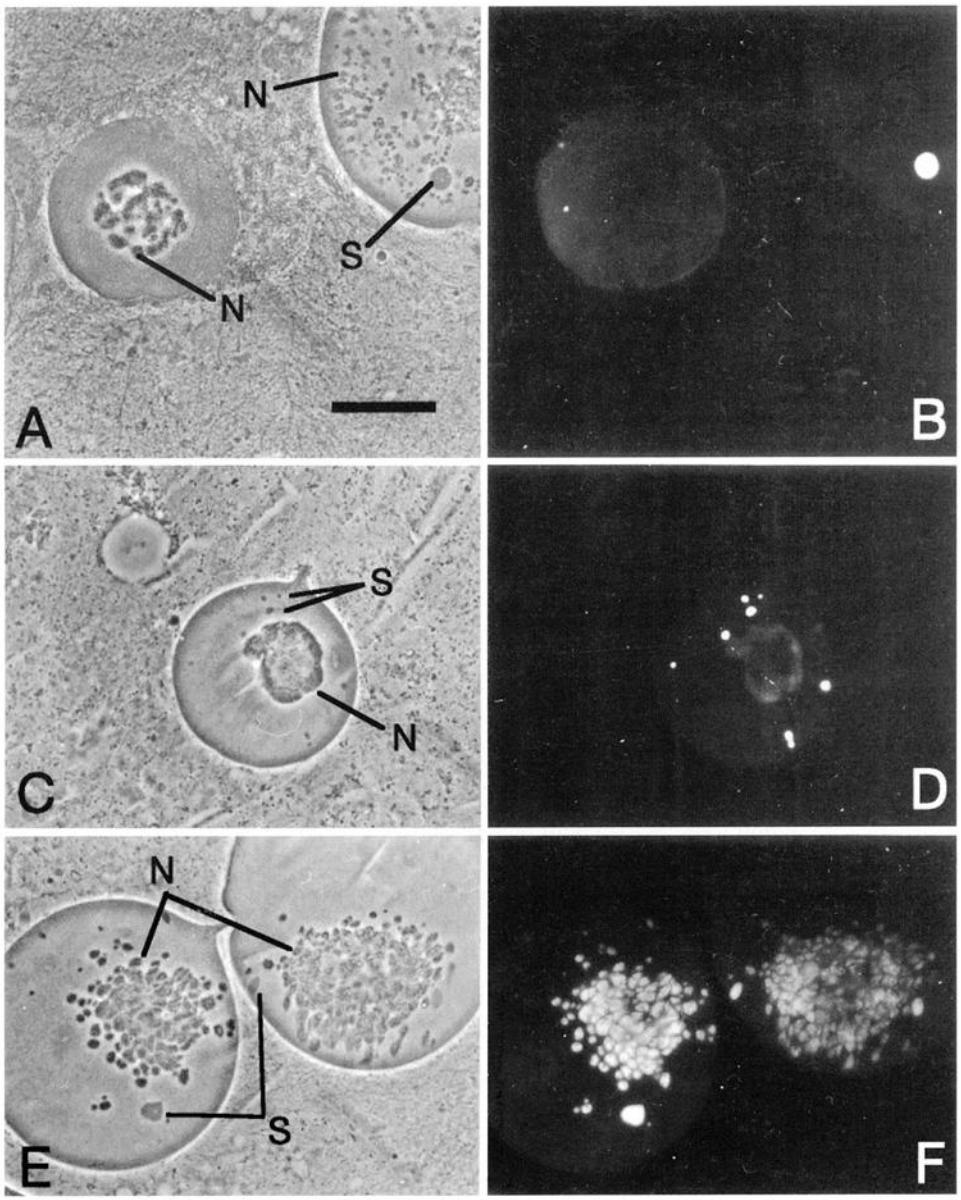


Fig. 1. A – a field containing nuclei of young previtellogenic and vitellogenic *Acheta domesticus* oocytes. Clumpy-shaped nucleolus and small spheres are present in the nucleus of young oocyte. In the vitellogenic oocyte, the sphere is the most prominent structure whereas the nucleolus has broken down into thousands of extrachromosomal nucleoli. (Phase contrast of squashed cricket ovarioles.) B – the same field viewed by fluorescence after staining with Y12 monoclonal antibodies. Only spheres are stained. C – a field containing young previtellogenic cricket oocyte nucleus. The major structure is the nucleolus. Small spheres are also present in nucleus. (Phase contrast of squashed cricket ovarioles.) D – the same field stained with K121 monoclonal antibodies. The spheres and nucleolus are stained. E – a field containing young vitellogenic oocytes. Nucleoli and spheres are visible. (Phase contrast of squashed cricket ovarioles.) F – the same as previous field viewed by immunofluorescence after staining with 17C12 monoclonal antibodies. Spheres and nucleoli are intensively stained. Bar = 20 μm.

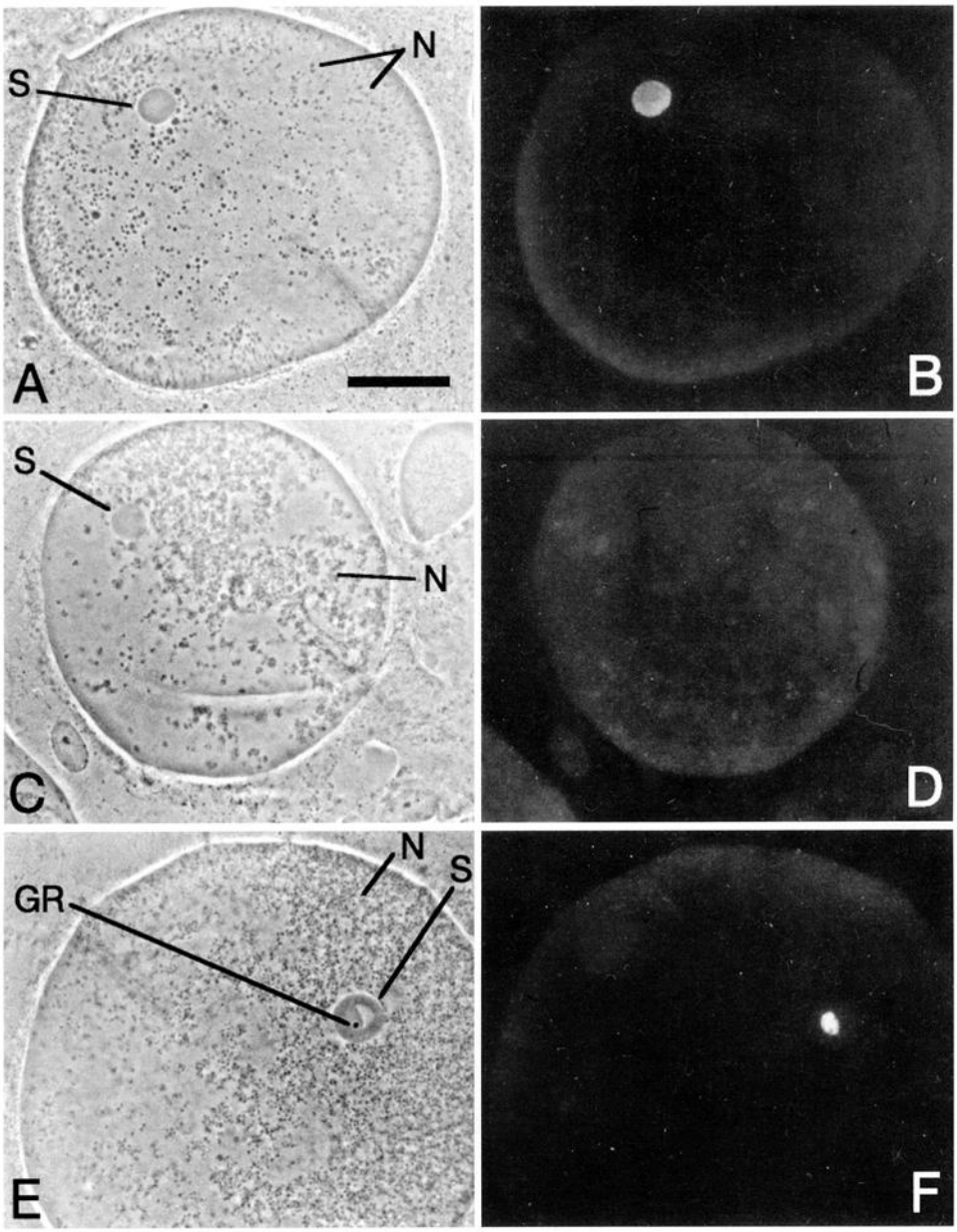
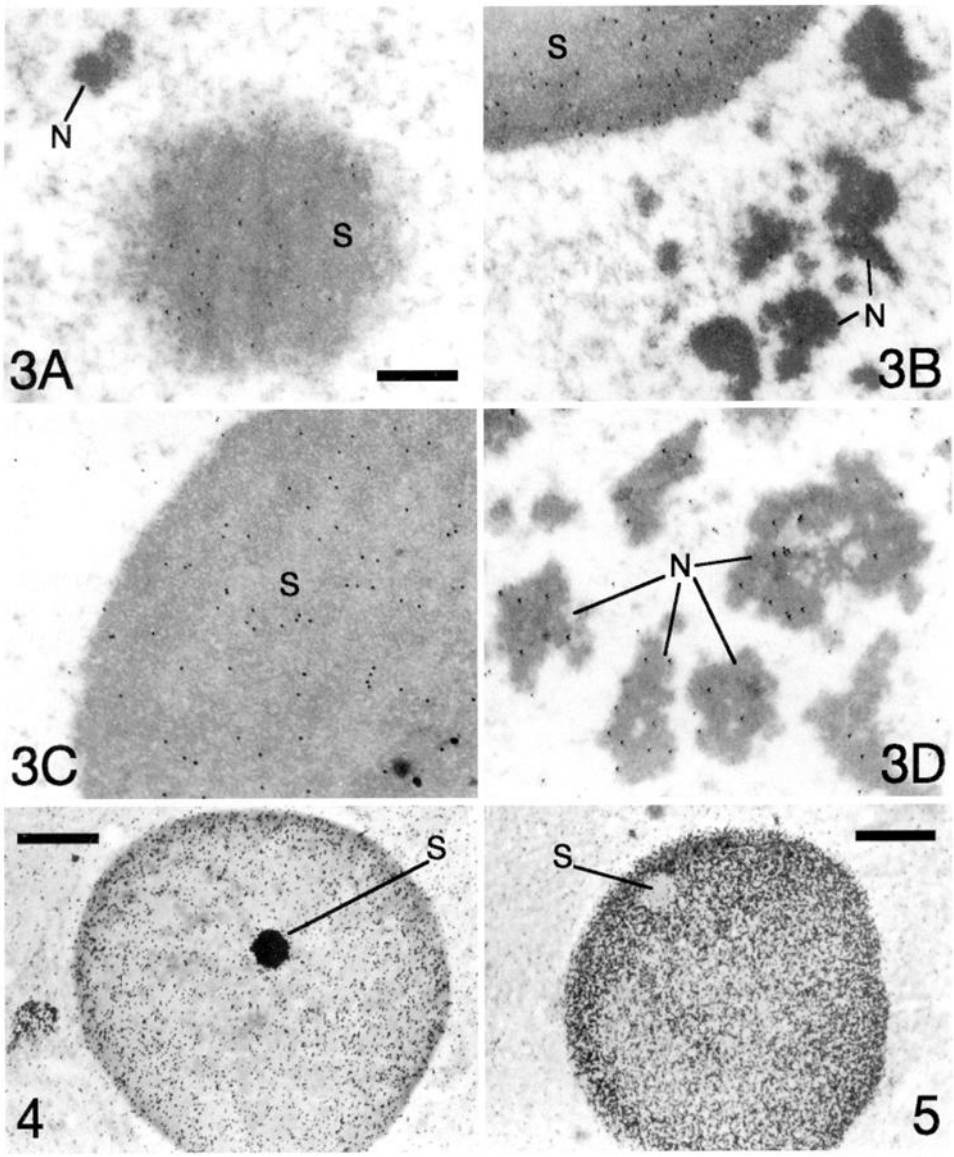


Fig. 2. A – a nucleus of large vitellogenic oocyte of *Acheta domesticus* containing sphere and numerous nucleoli. (Phase contrast of squashed cricket ovarioles.) B – the same field viewed after treatment with rabbit serum R288. The sphere is stained only. C – the nucleus of vitellogenic cricket oocyte with homogeneously structured sphere. (Phase contrast of squashed ovarioles.) D – immunofluorescent image of the same field after treatment with αSC35 monoclonal antibodies. The sphere is not stained. E – fragment of oocyte nucleus containing sphere with complex internal structure. The sphere contains a small granule and a vacuole. (Phase contrast of squashed cricket ovarioles.) F – the same field after treatment with αSC35 monoclonal antibodies. Small area surrounding granule is brightly stained. Bar = 20 μm.



Figs 3–5. 3A – stained ultrathin sections of *Acheta domesticus* oocyte nucleus after treatment with Y12 monoclonal antibodies as viewed using immunocytochemical electron microscopy. Only the sphere is stained. 3B – ultrathin section of cricket oocyte nucleus after treatment with K121 monoclonal antibodies. The sphere is labeled. 3C, 3D – ultrathin section of cricket oocyte nucleus after treatment with 17C12 monoclonal antibodies. Both spheres and nucleoli are stained. Bar = 0.5 μm. 4 – in situ hybridization of *Acheta domesticus* oocyte RNA with antisense U2 snRNA on squashed preparations of ovarioles. Sphere is very strongly labeled. Bar = 20 μm. 5 – incorporation of ^{3}H-uridine in *Acheta domesticus* oocyte nucleus after lengthy incubation period. Only sphere remains unlabeled on squashed preparation of cricket ovariole. Bar = 20 μm.

The treatment of cricket ovariole squashes with mAb αSC35 against SR-protein (non-snRNP splicing factor) did not reveal staining of spheres with homogeneous structure (Figs 2C–D). However, if spheres contained vacuoles and granules, material surrounding these granules was stained intensively (Figs 2E–F).

Electron microscopy study of cricket oocyte nuclei revealed that spheres consist of thin filaments (10–20 nm) and nucleoli are composed of tightly packed fibrils and are more electron dense than spheres. In some cases a connection between spheres and nucleoli has been revealed.

The treatment of ultrathin sections of *A. domesticus* ovarioles with mAbs K121 and Y12 resulted in only staining of spheres (Figs 3A–B). In contrast, mAb 17C12 reacted with nucleoli as well as with spheres (Figs 3C–D). In all cases, the staining was even; i.e. specific localization of antigens inside spheres was absent.

The in situ hybridization of U1, U2, U6 snRNA antisense probes with oocyte nuclei RNA on squashed preparations was completed to determine which snRNAs are present in spheres. Concentration of U2 snRNA in spheres (Fig. 4) was found to be much higher than levels evident in U1 and U6 snRNAs (Figures not shown). For instance, U2 snRNA could be revealed after 1–2 day exposure to probes while a two week exposure was necessary to reveal U1 and U6 snRNAs.

After longtime incubation of ovarioles in medium containing ^{3}H-uridine, the youngest previtellogenic oocyte nuclei were labeled intensively. However, we could not identify spheres on such preparations because of small sizes (0.2–1.5 μm) of spheres. In vitellogenic oocyte nuclei, spheres were the only unlabeled structures (Fig. 5). The absence of ^{3}H-uridine incorporation into spheres is the evidence that no pre-mRNA synthesis and splicing takes place in these organelles.

Nuclear organelles in *Tenebrio molitor* oocytes

Indirect immunofluorescent staining of *T. molitor* oocyte nuclei with mAbs Y12 and K121 revealed that all NB contain snRNPs (Figs 6A–I). Treating of squashes with mAb αSC35 against SR-protein revealed two groups of NB. One group showed bright staining while another remained unstained (Figs 7A–D). In *T. molitor* oocytes the karyosphere also shows strong staining with these mAbs (Figs 6A–B, D–E, 7A–B).

Oocytes of some species of Tenebrionidae lack true nucleoli (Gruzova & Batalova, 1979; Gruzova, 1979, 1982). This is confirmed by the absence of staining of *T. molitor* oocyte nuclei with mAb 17C12 against fibrillarin; in situ hybridization of rRNA probes with rDNA of *T. molitor* oocyte nuclei did not reveal rDNA in NB (Figure not shown).

Fig. 6 (see p. 400). A – the area of squashed vitellogenic oocyte nucleus of *Tenebrio molitor* with karyosphere and nuclear bodies (arrows). (Phase contrast.) B – the same area of nucleus viewed by fluorescent illumination after treatment with Y12 monoclonal antibodies. Karyosphere and nuclear bodies are strongly stained. C – cluster of nucleolar bodies treated with Y12 monoclonal antibodies from the same nucleus shown at higher magnification. D – the field containing a part of *T. molitor* oocyte nucleus with karyosphere and nuclear bodies (arrows). (Phase contrast of squashed nucleus.) E – the same field after treatment with Y12 monoclonal antibodies. Karyosphere and nuclear bodies are stained. F – the field containing karyosphere and one nuclear body. (Phase contrast of squashed nucleus). G – the same field after treatment with K121 monoclonal antibodies. Nuclear body and karyosphere material are stained. H – three nuclear bodies from oocyte nucleus spread. (Phase contrast.) I – the same nuclear bodies as viewed after treatment with K121 monoclonal antibodies. Bar: A, B, D, E – 20 μm; C, F, G, H, I – 10 μm.

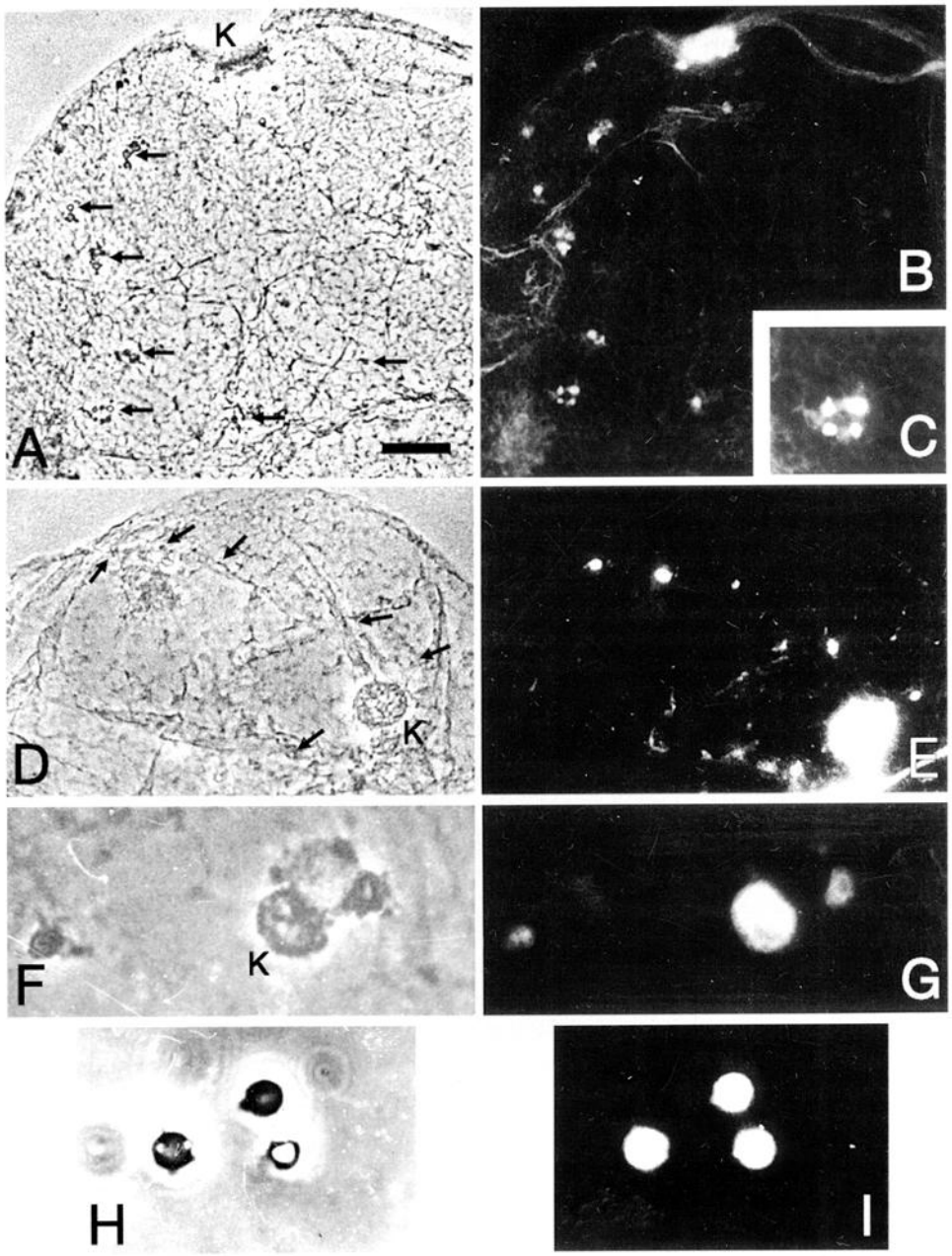


Fig. 6 (legend on p. 399).

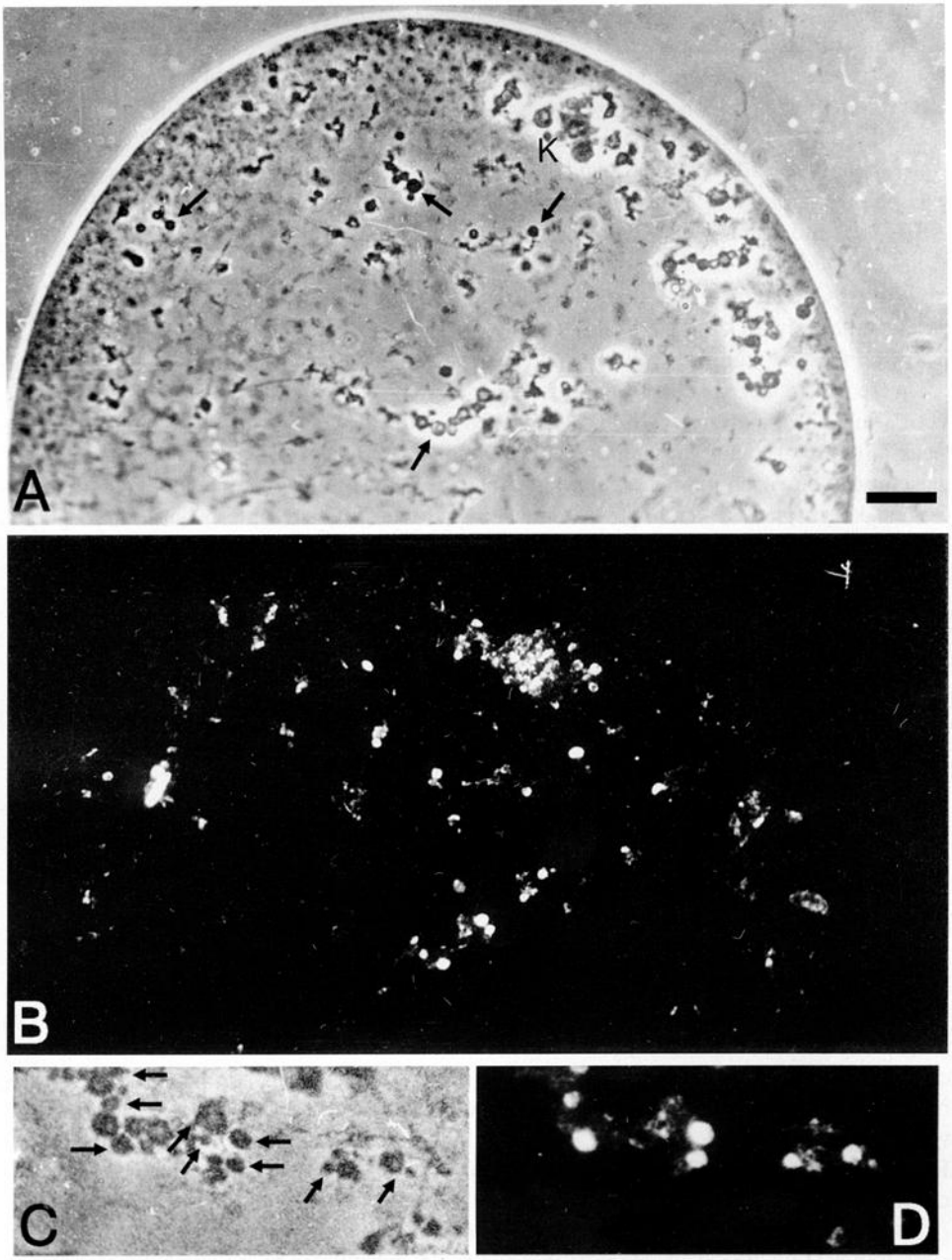


Fig. 7. An isolated nucleus of large vitellogenic oocyte of *Tenebrio molitor* containing karyosphere and nuclear bodies. A – phase contrast of nucleus before squashing and fixation. B – the same field of squashed and fixed nucleus as viewed after treatment with αSC35 monoclonal antibodies. Karyosphere and some nuclear bodies are stained. C – a field containing the fragment of nucleus from Fig. 6A. Nuclear bodies (arrows) clustering in karyoplasm are visible. (Phase contrast of squashed nucleus.) D – the same field as viewed after treatment with αSC35 monoclonal antibodies. Only some of nuclear bodies (pointed by arrows on Fig. 6C) are stained. Bar: A, B – 20 μm; C, D – 10 μm.

On an ultrastructural level, we found that there are two types of NB. Type I NB consists of small spherical structures (0.5–2.0 μm) and are composed of relatively large granules, about 30 nm in diameter, and fibrils that are approximately 30 nm in thickness. Type II NB (0.5–1.0 μm) consists of closely packed fibrils that are 10–15 nm in thickness. Sometimes Type I and Type II NB join to form a complex NB. These mentioned types of NB were all stained with mAbs Y12 and K121 (Figs 8A–B). As in the cricket spheres, staining was homogeneous, but Type II NB was stained more intensively then Type I NB. Using mAbs Y12 and K121, we have documented the presence of snRNPs in at least some NB of *T. molitor* oocytes.

mAb αSC35 stained both complex NB and Type I NB (Figs 8C–D). However, in these cases, only a few of the 30 nm fibrils and some of the large granules composing these NB were stained and significant portions of the granules remained unstained. Type II fibrillar NB were practically unstained.

In situ hybridization of U1 and U2 snRNA with RNA on ovariole sections result in weak labeling of young previtellogenic oocyte nuclei (Figures not shown). The labeling of these nuclei occurred mainly on chromosomes. In early vitellogenic oocyte nuclei that had formed karyospheres, labeling was detected in some NB, karyoplasm and karyosphere. The large vitellogenic oocyte nuclei were labeled intensively and almost evenly (Fig. 9A, B). These data suggest that snRNP accumulation takes place in the nucleus during oocyte growth.

Nuclei from young oocytes that had been incubated for a lengthy period in a medium containing ^{3}H-uridine were strongly labeled (Fig. 9C), but we could not detect NB. In vitellogenic oocytes, the area where the karyosphere is located was labeled by ^{3}H-uridine, while NB in karyoplasm remained unlabeled (Fig. 9D).

CONCLUSIONS AND DISCUSSION

Using light and electron immunocytochemistry we revealed that spheres from cricket oocyte nuclei and NB from mealworm oocytes contain Sm-epitope and trimethylguanosine cap of snRNA, which are the part of most snRNP involved in splicing of pre-mRNA.

Fibrillarin was found in spheres of *Acheta domesticus* oocytes as well as in nucleoli. This protein forms complexes with small nucleolar RNAs (snoRNAs) U3, U8 and U13 (Tyc & Steitz, 1989). It has been demonstrated that snoRNAs U3 and U8 are involved in processing of pre-rRNA (Peculus & Steitz, 1993). Spheres from cricket oocytes did not contain SR-protein (a major splicing factor), except in some stages in which it was possible to detect SR-protein in spheres with granules inside. In *Tenebrio molitor*, SR-protein was detected in only one type of NB. The bright staining of spheres by polyclonal serum R288 against human coilin allows us to conclude that these spheres contain coilin or coilin-like protein.

Spheres remained unlabeled after a longtime incubation of cricket ovarioles in medium containing ^{3}H-uridine. This suggests that synthesis and splicing of pre-mRNA does not occur in spheres.

In situ hybridization studies using antisense riboprobes revealed snRNAs U1, U2 and U6 in the spheres and karyoplasm of cricket oocytes and U1 and U2 snRNAs in NB and karyoplasm of mealworm oocytes. The concentration of U2 snRNA in spheres was much

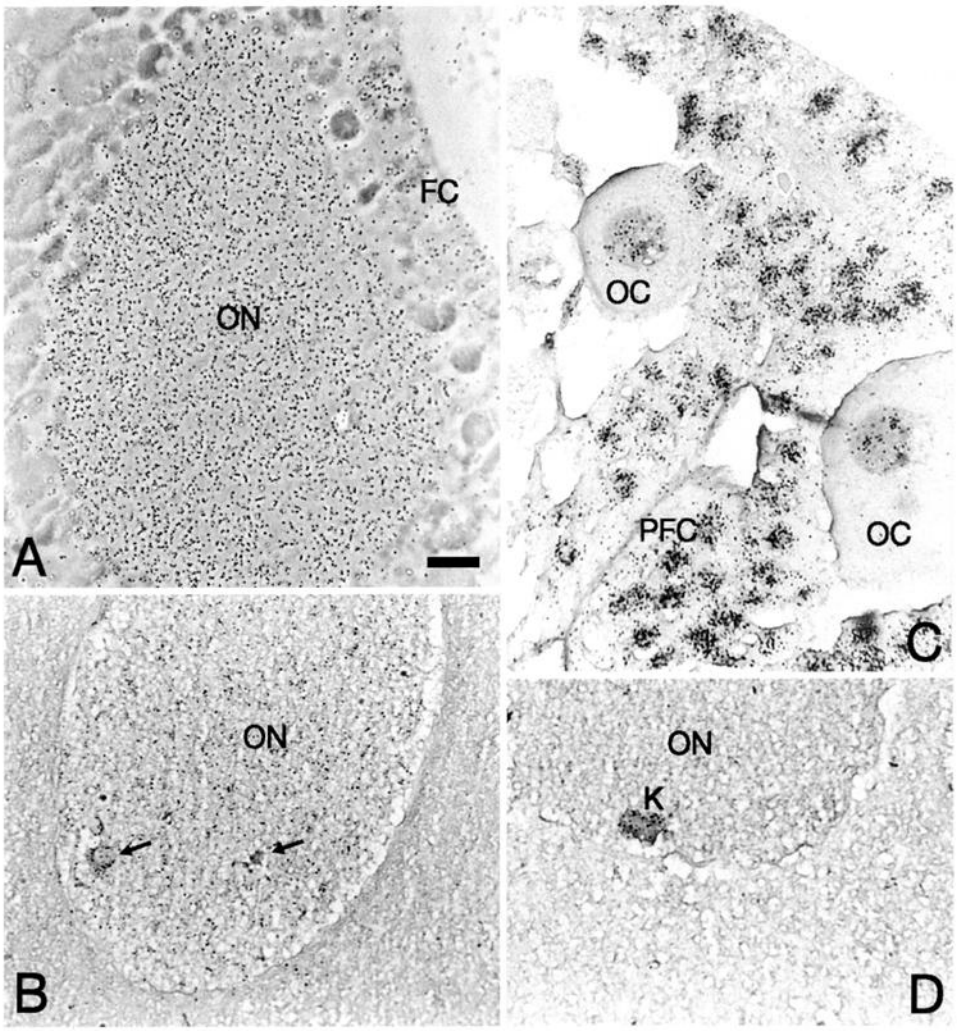


Fig. 8. Stained ultrathin sections of nuclear bodies of different types in *Tenebrio molitor* oocytes as viewed using immunocytochemical electron microscopy. A, B – complex body after treatment with Y12 monoclonal antibodies (A) and with K121 monoclonal antibodies (B). Both parts of these bodies are stained evenly after treatment with these antibodies. C, D – ultrathin sections of complex nuclear body (C) and Type I and Type II nuclear bodies (D) after treatment with αSC35 monoclonal antibodies. Fibrogranular material of Type I nuclear bodies is labeled. Type II nuclear body and similar fibrillar material of complex body remain unstained. Bar = 1 μm.

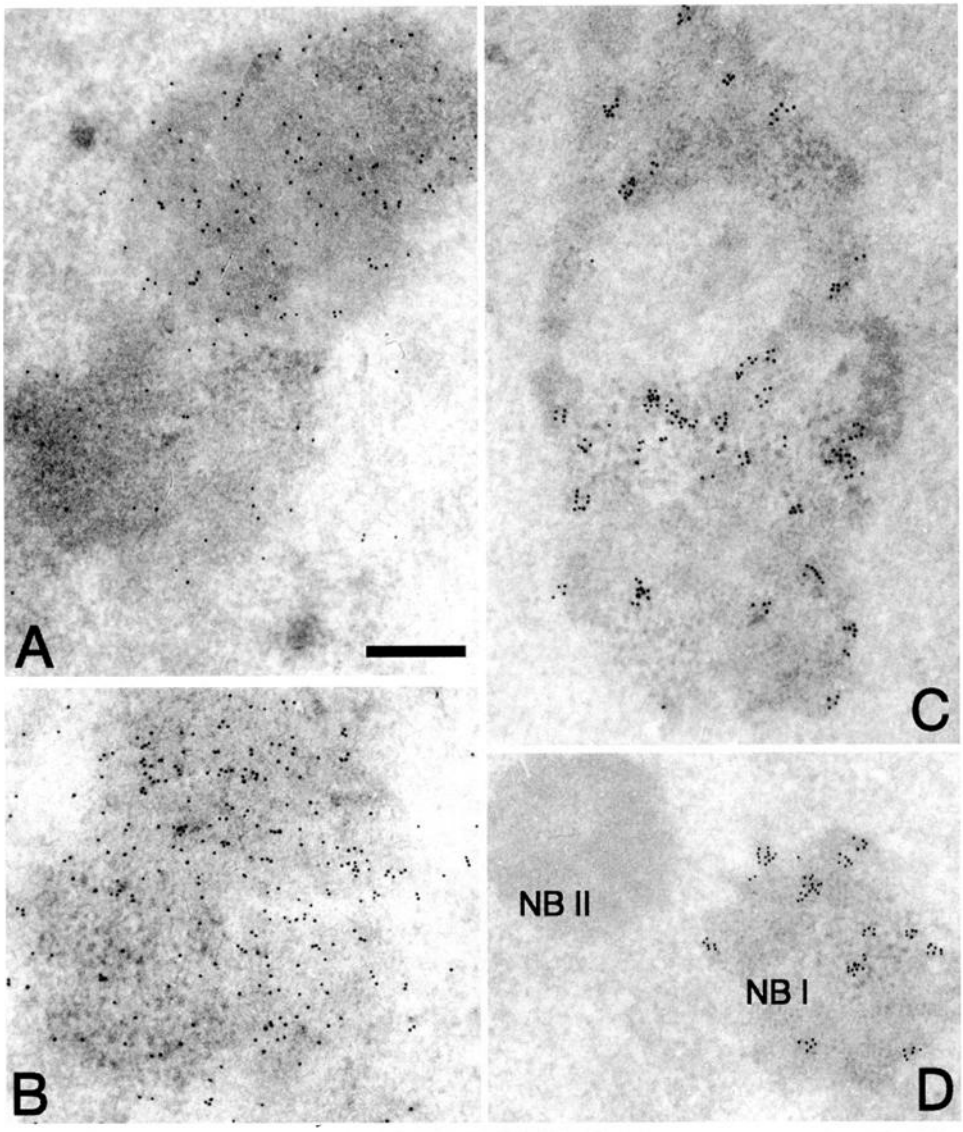


Fig. 9. A – nucleus of large vitellogenic oocyte from *Tenebrio molitor* after in situ hybridization with antisense U1 snRNA. Nucleus is heavily and evenly labeled. B – nucleus of the same stage after hybridization in situ with antisense U2 snRNA. Karyoplasm including nuclear bodies (arrows) is labeled. C – incorporation of ^{3}H-uridine in germarium of *T. molitor* ovariole. Nuclei of young oocytes and prefollicular cells are strongly labeled. D – incorporation of ^{3}H-uridine in vitellogenic oocyte nucleus. Note that the karyosphere is labeled. Bar = 10 μm.

higher than in U1 and U6 snRNAs. The high concentration of U2 snRNA has also been described in HeLa coiled bodies by Visa et al. (1993).

At present, a great amount of data regarding nuclear organelles containing factors of splicing of pre-mRNA, processing of pre-rRNA, and processing of 3′-end of histone mRNA is available. It has been shown that coiled bodies from somatic cell nuclei contain all major splicing snRNAs (U1, U2, U4, U5 and U6), snoRNA U3 and U7 snRNA involved in processing of 3′-end of histone mRNA, coilin and fibrillarin (Fakan et al., 1984; Raka et al., 1991; Carmo-Fonseca et al., 1991, 1992, 1993; Frey & Matera, 1995). It should be noted that coiled bodies do not contain SR-protein (one of the major splicing factors), thus splicing of pre-mRNA does not appear to take place in these structures. Prenucleolar bodies formed in vitro after adding DNA to *Xenopus laevis* egg extract contain all major snRNAs, U3 and U8 snoRNAs and U7 snRNA, as well as coilin and fibrillarin (Bauer et al., 1994). This indicates that the composition of prenucleolar bodies and coiled bodies is practically identical.

Two types of structures that contain snRNP other than lampbrush chromosomes have been described in amphibian oocyte nuclei (Gall et al., 1995). These are B-snurposomes, which contain all major snRNPs and SR-protein, and spheres, or sphere organelles. In amphibian germinal vesicles sphere organelles are complex structures; a sharp distinction occurs between the matrix of the central part that contains U7 snRNA and coilin, and two morphologically similar regions that contain splicing snRNPs – the inclusions and snurposomes on the surface. The sphere organelle contains all major snRNAs, U7 snRNA and coilin.

In *T. molitor* oocytes, we can identify one type of NB that contains SR-protein and snRNPs (NB I) and is analogous to amphibian snurposomes. Another type (NB II) also contains snRNPs but lacks SR-protein. Together, NB I and NB II form a complex structure, reminiscent of sphere organelles, in which NB II behaves as the central part of amphibian sphere organelles and NB I behaves as inclusions and snurposomes. Our preliminary data revealed staining of some NB with serum R288 on nuclear spreads (Alexandrova, 1993; Alexandrova et al., 1995), but we could not identify the type of stained NB. The lack of nucleoli in the oocyte nuclei of *T. molitor* explains the absence of fibrillarin in NB.

Thus, we conclude that coiled bodies, prenucleolar bodies, amphibian sphere organelles, spheres from cricket oocytes and NB complexes from *T. molitor* oocytes (with some exception) possess essential homology; these structures contain coilin or the coilin-related protein and a set of snRNPs. All nuclear organelles mentioned above probably represent one universal nuclear structure, the possible function of which is the assembly and organization of snRNP complexes for pre-mRNA splicing, rRNA processing and histone pre-mRNA 3′-end formation.

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