The Karyosphere Capsule in *Tribolium castaneum* Oocytes

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Abstract—The structure and composition of the karyosphere (karyosome) capsule of the laboratory insect, *Tribolium castaneum*, were studied with the use of electron and immunoelectron microscopy. Eight stages that characterized the period of oocyte growth have been established basing on the study of nuclear structure dynamics. At the diplotene stage, *T. castaneum* oocyte chromosomes are being united early to form a compact karyosphere; however, prominent chromatin condensation does not occur at the same time. The process of karyosphere formation is accompanied by the development of a spacious extrachromosomal capsule surrounding chromatin. The capsule consists of a fibrous material of different morphological types. The most prominent molecular components of *T. castaneum* karyosphere capsule are represented by the proteins of nuclear matrix, including F-actin and lamin B. Apart from the structural proteins, immunocytochemical approach allowed revealing Sn proteins of small nuclear (sn) RNPs and “mature” snRNAs with 2,2,7-trimethyl guanosine (TMG) cap at the 5’-end of their molecules. These data may serve as a base for further broadening of the conception about the functions of the karyosphere capsule as a specialized oocyte nuclear domain. We believe that *T. castaneum* karyosphere capsule plays not only a structural role, but may be involved directly in the processes related to gene expression.

Keywords: *Tribolium castaneum*, oocyte nucleus, extrachromosomal nuclear domains, karyosphere, karyosphere capsule, electron microscopy

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In many animals, karyosphere (karyosome) formation is a unique feature of oocyte (rarely spermatoocyte) nuclear structure, as compared with somatic cells. The karyosphere appears as a result of concentration of all germ cell chromosomes in the limited nuclear volume, forming a complex structure that may include a material of the nucleolus and/or different extrachromosomal nuclear bodies (Gruzova et al., 1995). The karyosphere has been described in more than 120 species that belong to 4 phyla of animal kingdom (Gruzova and Parfenov, 1993; Gruzova et al., 1995). Amongst invertebrates, karyosphere is the most vivid and typical in growing oocytes of insects with meroistic ovaries (for the structure of insect female gonads, their evolution and the features of oogenesis, see the reviews by Büning, 1994; Biliński, 1998).

Together with actively functioning nurse cells (trophocytes), karyosphere forms at the beginning of diplonte and exists until the end of the period of oocyte growth.

The molecular mechanisms of karyosphere formation are poorly known; however, it is likely that phosphorylation play an essential role in this process. For example, specific phosphorylation of Thr-119 in the molecules of histone H2A is required for karyosphere formation in *Drosophila* oocytes (Ivanovska et al., 2005). Phosphorylation of BAF (barrier to autointegrator factor) by the conserved kinase NHK-1 (Vrk-1 in *Drosophila*) also impacts significantly on the process of karyosphere formation (Lancaster et al., 2007). The signal pathway blocking meiosis at the meiotic checkpoint reduces NHK-1 activity and prevents further oocyte nucleus reorganization including karyosphere formation (Lancaster et al., 2010). Another evolutionarily conserved kinase, SRPK, also regulates karyosphere formation in *Drosophila*, since normal chromosome gathering during karyosphere formation is disrupted in sterile srpk mutant females (Loh et al., 2012).

The general character of karyosphere morphogenesis looks similar in oocytes of various insects and consists in progressive chromatin condensation and production of an extrachromosomal material of different types. At the same time, morphological characteristics
of the karyosphere are species-specific. The most vivid characteristics are the extent of chromatin condensation and, in some cases, the presence of a peculiar extrachromosomal capsule surrounding chromatin. When the capsule exists, karyosphere was being called a peculiar “nucleus in the nucleus,” stressing the significant isolation of chromosomes from the rest of the nucleoplasm (Gruzova and Parfenov, 1993).

Amongst insects with meroistic-polytrophic ovaries, a well-developed capsule characterizes the oocytes of neuropterans (Gruzova et al., 1972; Rübsam and Büning, 2001). On the contrary, it lacks in the studied representatives of the orders Mecoptera (Batalova et al., 2005) and Phthiraptera (Żelazowska and Jaglarz, 2004). In a complex karyosphere capsule that is formed, in particular, by the derivatives of synaptonemal complexes develops in mosquito oocytes (Fiil and Moens, 1973; Fiil, 1974). However, karyosphere lacks a capsule in oocytes of higher dipterans, including Drosophila, Musca, Calliphora, Sarcophaga, and Glossina (Bier et al., 1967; Mahowald, Tiefert, 1970; Huebner et al., 1975; Cardoen et al., 1986).

Amongst the studied insects with meroistic-telotrophic ovaries, which the representative of Tenebrionidae Tribolium castaneum that has been explored in the present study belongs to, karyosphere capsule develops in oocytes of Silphidae (Matuszewski et al., 1977) and weevils (Curculionidae) (Świątek, 1999; Nardon, 2006). In tenebrionids, karyosphere capsule is present in oocytes of Blaps leithera, B. moritisaga, Gnapter spinimanus (Gruzova and Batalova, 1979; Gruzova, 1979, 1982), Tenthyria nomas taurica (Alexanderova, 1992) and T. castaneum (Bogolyubov et al., 2013, and the present work), but it lacks in Tenebrio molitor (Bogolyubov et al., 2000; Bogolyubov and Parfenov, 2001). Amongst the representatives of other families of polyphagous beetles, karyosphere capsule does not develop in oocytes of the seven-spotted lady beetle, Cocinella septempunctata (Kozhanova and Pasichnik, 1979), the seed beetle, Bruchidius obtectus (Büning, 1980), and outside the Coleoptera-Polyphaga in the snakely, Raphidia flavipes (Büning, 1980).

A functional role of the karyosphere capsule as well as its molecular composition is not fully elucidated (see Discussion).

Here we continue our studies, initiated earlier (Bogolyubov et al., 2012, 2013) to explore the structure, molecular composition and morphodynamics of nuclear structures, including the karyosphere and its capsule, during T. castaneum oogenesis. During the last years, the red flour beetle, T. castaneum, became attractive for the researchers as a perspective model organism, because the genome of this species has been fully sequenced, aligned and is available for the research community (Tribolium Genome Sequencing Consortium, 2008). These beetles are harmful pests and also may cause an allergic response in humans, but cannot disperse diseases and damage wooden constructions or furniture. In comparison with Drosophila, the traditional laboratory insect, Tribolium displays a number of undoubted advantages as the model organism (Peel, 2009). For example, Drosophila is characterized by several evolutionarily specialized features, non-typical for Arthropoda as a whole. Small genome size and a set of specific morphological, physiological, behavioral and ecological features are among these peculiarities.

It is undoubted, that the new model organism has to be characterized comprehensively. However, available data on T. castaneum oogenesis and its oocyte structure are being remained scanty to the date. Only the microanatomy of the female gonads of this species has been described in details; differentiation of oocytes and nurse cells was also traced (Trauner and Büning, 2007). Other studies carried out on the T. castaneum female reproductive system have established the influence of the heat shock on the ovary development and expression of hsp83 gene, homologous to human hsp90 gene (Xu et al., 2009, 2010a). Several transcription factors, which play a significant role in T. castaneum reproduction and fertilization, has also been recognized (Bitra and Palli, 2010; Xu et al., 2010b). Finally, several studies were devoted to investigations of hormonal regulation of reproduction, ovary development and vitellogenin synthesis in females of this species (Parthasarathy et al., 2010a, 2010b; Parthasarathy and Palli, 2011). Ahead of our works (Bogolyubov et al., 2012, 2013, present study), nothing was known about the features of the structure of the T. castaneum oocyte nucleus.

MATERIALS AND METHODS

T. castaneum (Herbst) (Coleoptera-Polyphaga, Tenebrionidae) was cultured in the darkness at +28°C. The gonads of adult females were isolated in the physiological medium for insects (0.75% NaCl, 0.035% KCl, 0.021% CaCl$_2$). Non-fixed nuclei isolated from the oocytes of different stages of growth were observed in a fluorescent microscope Leica DM IRB equipped with Nomarski optics (DIC). 1.0 µg/mL 4,6-diamidino-2-phenylindole (DAPI) was added in the medium.

For routine electron microscopy, single ovarioles (anatomical units of ovaries) were fixed at +4°C in 2.5% glutaraldehyde (Polyscience, USA) prepared in 0.1 M cacodylate buffer, then post-fixed in 4% OsO$_4$ for 1.5 h at room temperature and embedded in Spurr (Ted Pella, Inc., Redding, United States). Ultrathin sections were contrasted with uranyl acetate and lead citrate. The sections were examined in electron microscopes Libra 120 or JEM-7A at 80 kV.

For immunoelectron microscopy, ovarioles were fixed in PBS containing 0.5% glutaraldehyde and 4% formaldehyde, freshly prepared from paraformaldehyde, for 2 h at room temperature, then in 2% formaldehyde in PBS overnight at +4°C. After rinsing in PBS