The Balbiani body is a non-membrane bound complex of organelles positioned at one side of the oocyte nucleus (germinal vesicle). It consists mostly of mitochondria and characteristic aggregates of fibrillo-granular nuage material but also Golgi complexes, cisternae and/or vesicles of endoplasmic reticulum and even cytoskeletal fibers. Despite the fact that the morphology as well as morphogenesis of the Balbiani body have been intensely studied in numerous animal species, the role of this organelle assemblage is still under debate. It has been suggested that the Balbiani body is involved in the delivery of macromolecules (localized mRNAs) and organelles (mitochondria, germinal granules) to the germ plasm, lipidogenesis, and selective elimination of defective mitochondria. Such functional variability raises an intriguing question: which of the suggested Balbiani body function is phylogenetically ancestral, and which are secondary and characteristic only for a given derived animal group? It is to the point to add here that the morphology and composition of the Balbiani bodies of analyzed invertebrate and vertebrate species (including model species such as *Xenopus laevis*, *Danio rerio* and *Drosophila melanogaster*) are highly variable and usually show features characteristic for a specific group (taxon). If so, the second question can be asked: are the functions of the Balbiani body described with the use of model organisms universal for the entire animal kingdom or rather specific for a certain animal group/s?

Therefore, the aim of the present doctoral dissertation was to determine the ultrastructure and mechanisms controlling morphogenesis (formation, gradual growth and break down) of the Balbiani body and the role of this complex in the multiplication and selective elimination of mitochondria during oogenesis. To achieve this goal, a series of analyses and experiments were performed. Female gonads of five bush cricket species (Tettigoniidae) were used in morphological and comparative studies: *Meconema meridionale*, *Metrioptera brachyptera*, *Pholidoptera griseoaptera*, *Conocephalus fuscus* and *Leptophyes albovittata*. As the colony of *Meconema meridionale* has been maintained throughout the duration of the research, detailed studies and experimental analyses were carried out using this species only. Standard and recently developed methods of light, electron and confocal microscopy were employed in the studies. Functioning of the Balbiani body was analyzed with histochemical and immunohistochemical methods using variable molecular markers.

Analyses of serial semithin and ultrathin sections revealed that in Tettigoniidae, the Balbiani body is relatively large and morphologically complex. It arises as early as in the meiotic oocytes, in a stage referred to as the bouquet stage, intensively grows during previtellogenesis and disintegrates (breaks down) with the onset of the accumulation of reserve materials, i.e. vitellogenesis. Despite some morphological differences, the Balbiani bodies

of all studied species are built of the same "set" of organelles: numerous mitochondria, short cisterns and small vesicles of the endoplasmic reticulum, Golgi complexes and irregular aggregates of nuage material. The Balbiani bodies of the studied species share also an important feature, i.e. close relationship of mitochondria and nuage aggregates. Mitochondria remaining in direct contact with the nuage or located in its vicinity are often elongated, bifurcated, dumbbell shaped or merged in characteristic pairs. These findings imply that nuage triggers biogenesis of mitochondria. Results of 1,6-hexanediol treatment and PROTEOSTAT staining jointly indicate that in *Meconema meridionale*, as in the model species *Xenopus* and *Danio*, the formation of the Balbiani body involves proteins with intrinsically disordered regions, and therefore, represents a typical biomolecular condensate.

Computer aided 3-dimensional reconstructions of the organization of *Meconema* Balbiani body showed that the mitochondria within this complex are interconnected constituting an intricate network. Analyses with a thymidine analogue BrdU showed that within this network intensive mtDNA replication and consequent mitochondrial multiplication take place. Furthermore, the analyses of mitochondrial membrane potential, as well as the immunohistochemical localization of proteins involved in mitochondrial division (Drp1) and autophagy (ATG5, LC3) indicated that non-functional mitochondria (with reduced membrane potential and/or abnormal morphology) are separated from the network and eliminated in the ooplasm *via* autophagy (mitophagy).

Immunolocalization of α-tubulin showed that the previtellogenic oocytes of *Meconema meridionale* contain a complex system of microtubules that penetrates the entire ooplasm. Ultrastructural analyses of colchicine treated oocytes indicated that this system is not involved in the formation of the Balbiani body, but participate in the positioning of the nuage aggregates in the immediate vicinity of the germinal vesicle. Finally, the hybridocytochemical localization of Drp1 mRNA, performed on control and colchicine treated oocytes sections, revealed that the above-mentioned system of microtubules is responsible for the uniform distribution of Drp1 mRNA throughout the expanding ooplasm of quickly growing previtellogenic oocyte. It seems likely, that this process ensures the even distribution of Drp1 protein, and consequently the synchronization of mitochondrial divisions in the whole oocyte cytoplasm.

In conclusion, the results of the presented studies imply that the preferential transmission of wild-type mitochondria to the offspring represents an ancestral function of the Balbiani body. Other, previously proposed, functions of this organelle assemblage presumably evolved secondarily in certain derived vertebrate and invertebrate groups, such as fishes (*Danio*), frogs (*Xenopus*) and holometabolous insect (*Drosophila*). Therefore, the results

obtained in the present dissertation support the previously voiced idea that the findings and conclusions drawn from studies conducted on the model species only, cannot be extended to all animal taxa.

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