

Mitochondria are semiautonomous organelles that contain their own genome, termed the mitochondrial DNA (mtDNA), and protein synthesis machinery. The most important function of mitochondria is to supply cells with metabolic energy in the form of ATP molecules generated by oxidative phosphorylation. Because synthesis of ATP is accompanied by generation of harmful reactive oxygen species (ROS), mtDNA is particularly vulnerable to mutations and lesions. It is widely accepted that organisms reproducing asexually accumulate growing number of irreversible deleterious mutations over generations. This phenomenon, known as Müller's ratchet, affects the viability of cells, and consequently whole organisms. As in most animal taxa, mitochondria are inherited from one generation to the next exclusively maternally, i.e. asexually, their mtDNA must escape from the ratchet or (alternatively) must be protected against this impairment. Although the mechanism of such protection is still not fully understood, it has been suggested that it must involve two processes: (1) selective elimination of defective (containing mutated mtDNA) mitochondrial units, and (2) transmission of only accurate (devoid of mutations) mtDNA from one generation to the next. Experimental data indicate that mitochondrial selection in developing oocytes is associated with the Balbiani body, a non-membrane bound assemblage of various cytoplasmic constituents as mitochondria, elements of endoplasmic reticulum and accumulations of fibrillo-granular electron dense material, termed the nuage. Although the Balbiani body has been reported in the oocytes of several animal groups (millipedes, spiders, insects, amphibians and mammals) its formation, composition and ultimate fate have only been analyzed in a handful of model species, e.g. frog, *Xenopus laevis*. As a result, the exact relationships between the Bb constituents, processes of its formation and fragmentation or functions are poorly understood. Interestingly, molecular studies have demonstrated that in *Xenopus* formation of the Bb is initiated by amyloid-like self-assembly of proteins with prion-like domain that resembles self-assembly of amyloid proteins in Alzheimer's diseased brain cells. In this context, the gradual dispersion of the Bb, which takes place under physiological conditions in all examined animal species, is of special interest.

The aim of the proposed project is to gain further insight into the mechanisms implicated in the formation and disintegration of the Bb and concurrent selection of mitochondria. Moreover, the participation of selected organelles (e.g. lysosomes, nuage), cytoskeletal elements and dynamin related proteins in these processes will be analyzed. First, I will test whether the morphogenesis of the Bb in basal insect species follows the pattern described in *Xenopus*. In the second part, I would like to verify the idea that nuage triggers mitochondrial multiplication. Next, to define the role of the cytoskeleton in the formation and dispersion of the Bb, I will analyze the distribution of microfilaments and microtubules in successive stages of oogenesis. Additionally, I will analyze the effects of cytoskeletal disrupting drugs on Bb formation and fusion/division of its mitochondria. In the last part, I will study the role of lysosomes and proteins involved in mitochondrial dynamics (mitofusins, dynamins) in Bb morphogenesis and multiplication of germline mitochondria. I expect that my studies will provide answers to two more general evolutionary questions: Are the Bb functions universal for the entire animal kingdom or unique only for a particular lineage? Which of the Bb functions are ancestral and which evolved secondarily?

In the project, both standard and recently developed methods of light, electron and confocal microscopy will be used. The organization and functions of the Balbiani body will be studied with cytochemical and immunocytochemical methods using variable molecular markers. A representative of basally branching insect taxon (Orthoptera), the bush cricket *Meconema meridionale* will be exploited as a research model, as it is frequently found, not protected or endangered and it can be collected without any specific permissions.