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Arthropod Structure & Development 36 (2007) 317–326

ARTHROPOD STRUCTURE & DEVELOPMENT

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The Balbiani body in the oocytes of a common cellar spider, *Pholcus phalangioides* (Araneae: Pholcidae)

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Received 12 October 2006; accepted 25 January 2007

Abstract

Previtellogenic oocytes of a common cellar spider, *Pholcus phalangioides*, contain a single aggregation of organelles referred here to as the Balbiani body. It is a well defined ooplasmic structure predominantly composed of fine granular nuage, RNA rich material but comprising also mitochondria, vesicles of endoplasmic reticulum and stacks of Golgi cysternae. The Balbiani body originates early during previtellogenesis in the form of a cap-shaped mass in juxtaposition to one pole of the oocyte nucleus. During later stages of previtellogenic growth the Balbiani body translocates as a single body towards the ooplasm periphery. The results presented indicate that Balbiani body translocation is cytoskeleton independent. Balbiani body repositioning does not result in the localization of its components to any distinct, asymmetrically situated region of the ooplasm but, instead, ends up with their even dispersion in the oocyte cortex. The Balbiani body in *Pholcus* does not seem to be implicated either in germ cell determination or organelle inheritance. Its homology with similar organelle accumulations in the oocytes of other species is discussed.

Keywords: Organelle accumulations; Nuage; Oogenesis; Spider

1. Introduction

One of the most spectacular events during oogenesis is oocyte growth, which proceeds in two distinct phases. Previtellogenic growth is conditioned by the accumulation of organelles and macromolecules. During vitellogenesis, the oocyte is filled with various reserve materials such as glycoprotein yolk spheres, lipid droplets and glycogen. Stored in the ooplasm during oogenesis, all these resources are used to support early embryonic development.

It has been evidenced that during previtellogenesis organelles and macromolecules (RNAs and proteins) may be specifically targeted and localized to distinct regions of the ooplasm (King et al., 2005; Kloc and Biliński, 2003; Kloc and Etkin, 2005). Since at least some of these molecules are cell fate determinants, their subsequent asymmetric inheritance by the blastomeres during early embryonic development plays a crucial role during early cell differentiation processes and developmental determination. Since the pioneer XIX century work of Wittich, Van Bambeke, Balbiani and others (for historical background see: Guraya, 1979) it has been known that the organelles and their accompanying material may form well defined, distinct accumulations in the ooplasm. These accumulations were originally termed ''yolk nuclei'' and later ''Balbiani vitelline bodies''. They were repeatedly reported in various invertebrate and vertebrate species. Comparative histological and TEM analyses revealed that they may show significant structural complexity. Since the Balbiani bodies (Bbs) usually consist of fibro-granular nuage material associated with mitochondria, they were often referred to as ''mitochondrial clouds''. The Bbs also contain vesicles or cysternae of endoplasmic reticulum and Golgi complexes, but may also contain several other organelles and inclusions, like ribosomes, annulate lamellae, lipid droplets and multivesicular bodies. At least in

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doi:10.1016/j.asd.2007.01.004

the oocytes of *Xenopus* and *Drosophila*, Bbs were also found to associate with the fusome, a specialized cytoplasmic structure that arises during incomplete cytokineses within the intercellular bridges that connect the germ cells in a cluster (Cox and Spradling, 2003; Kloc et al., 2004b; for a review on Bb structure, formation and significance in various animal species see: Kloc et al., 2004a).

In several animal species studied so far, the production and assemblage of the Bb constituents follow a similar pattern during oogenesis. Early during oogenesis, prior to the previtellogenic phase of oocyte growth, components of the Bbs are aggregated adjacent to the oocyte nucleus (germinal vesicle – GV) either in the form of regular spherical structures or of cap-shaped accumulations. Subsequently, during previtellogenesis, the Bb moves away from the GV towards the oocyte cortex. In a few well known situations (e.g. in *Xenopus*) this movement is directed, so the Bb eventually occupies a distinct, morphologically distinguishable domain within the oocyte (Kloc and Etkin, 1995; Kloc et al., 1998). The presence of this asymmetrically located domain marks clearly the polarity of the oocyte.

Although Bbs may exhibit conspicuous structural variety even among closely related species, some authors considered them as homologous structures. Initially, the estimation of their homology was merely based on histological characteristics and the analysis of their behavior during oogenesis. With the advent of molecular techniques, in at least a few cases ''structural identification'' was substantially supplemented by ''identification'' of homologous molecular markers. It appeared that Bbs contain several characteristic macromolecules (mostly RNAs) (reviewed in Kloc et al., 2004a). The assessment of homology was extended to the molecular level and thus significantly facilitated.

Several lines of evidence indicate that in such model organisms, as, e.g. *Xenopus*, Bbs comprise determinants that may specify the germ cell fate (Forristall et al., 1995; Kloc et al., 1998; MacArthur et al., 2000; Madacho et al., 2005). Recent studies, using a wide range of contemporarily available methods, clearly showed that Bbs not only accumulate germ cell determinants and organelles and form a means for their sorting and maturation, but were also vehicles used during oogenesis for coordinated and directed transport of germ plasm constituents and organelles to the place of their ultimate localization within the ooplasm (reviewed in Houston and King, 2000; Kloc et al., 2001; Cox and Spradling, 2003; Wilk et al., 2004).

The wealth of data obtained in the extensive studies on model organisms contrasts with the limited knowledge we have on the molecular composition and behavior of Bbs in other animal groups. Although spiders and myriapods were the first in which the Bbs were identified, contemporary data on their composition, behavior and ultimate fate during oogenesis are nearly non-existant. The most recent and detailed references were made in a comprehensive review published nearly 30 years ago (Guraya, 1979). Although Guraya devoted the whole chapter of his review to ''yolk nuclei'' in spider oocytes, ultrastructural and histochemical data presented therein are currently outdated. Thus reliable comparative considerations on the possible homology of these structures with those that have been so extensively studied in organisms such as *Xenopus* or *Drosophila*, would be hard to give. To make these comparisons possible we decided to describe in detail the structure and behavior of the Bbs in spider oocytes employing more modern methods. Special emphasis has been put on the detection of at least some of those components that are considered as characteristic for ''model Balbiani bodies''. We believe that our data will provide a basis for future, more precise, molecular analyses of the structure and function of ''ooplasmic accumulations'' and their significance in oogenesis and embryogenesis in chelicerates.

In this paper we present the results of morphological and cytochemical analyses of the structure and behavior of the Bb in the oocytes of the cellar spider, *Pholcus phalangioides*. Based on the data obtained we speculate on its homology with similar structures described in other species.

2. Materials and methods

Since *P. phalangioides* is a common synanthropic spider, young and adult females for the use of this study were collected indoors in several different places.

2.1. Light and transmission electron microscope

For histological and ultrastructural observation, ovaries were dissected and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH = 7.4) for longer periods. For gross morphology analysis, fixed ovaries were viewed with an Olympus BHS light microscope equipped with Nomarski optics. After fixation with glutaraldehyde, material was repeatedly rinsed with phosphate buffer and for better tissue preservation and contrast was subsequently postfixed in the mixture containing 1% osmium tetroxide and 0.8% potassium ferrocyanide (according to McDonald, 1984). After dehydration in acetone series the material was embedded in Epon 812 (Serva, Heidelberg, Germany). Semithin sections (0.6 μm) were stained with 1% methylene blue in 1% borax and examined with an Olympus BHS light microscope. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in a Zeiss EM 900 at 80 kV.

2.2. Histochemical and immunohistochemical analyses

The ovaries were dissected and fixed in 4% formaldehyde in phosphate-buffered saline PBS (NaCl, 137 mM; KCl, 2.7 mM; Na_2HPO_4, 8 mM; KH_2PO_4, 1.5 mM). After a few rinses with the PBS the material was proceeded either for sectioning or whole mount staining.

2.2.1. Detection of DNA and RNA on semithin sections

The material was rinsed with PBS, dehydrated in an ethanol series and embedded in acrylic resin Histocryl (Agar, Stanstead, UK). Differential detection of nucleic acids was achieved by staining semithin histocryl sections (1.5 μm) sequentially with DAPI (4′,6 diamidino-2 phenylindole dihydrochloride)