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Yolk nucleus – The complex assemblage of cytoskeleton and ER is a site of lipid droplet formation in spider oocytes

Izabela Jędrzejowska*, Janusz Kubrakiewicz

Department of Animal Developmental Biology, Zoological Institute, University of Wrocław, Sienkiewicza 21, 50-335 Wrocław, Poland

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ABSTRACT

Oocytes (future egg cells) of various animal groups often contain complex organelle assemblages (Balbiani bodies, yolk nuclei). The molecular composition and function of Balbiani bodies, such as those found in the oocytes of *Xenopus laevis*, have been recently recognized. In contrast, the functional significance of more complex and highly ordered yolk nuclei has not been elucidated to date. In this report we describe the structure, cytochemical content and evolution of the yolk nucleus in the oocytes of a common spider, *Clubiona* sp.. We show that the yolk nucleus is a spherical, rather compact and persistent cytoplasmic accumulation of several different organelles. It consists predominantly of a highly elaborate cytoskeletal scaffold of condensed filamentous actin and a dense meshwork of intermediate-sized filaments. The yolk nucleus also comprises cisterns of endoplasmic reticulum, mitochondria, lipid droplets and other organelles. Nascent lipid droplets are regularly found in the cortical regions of the yolk nucleus in association with the endoplasmic reticulum. Single lipid droplets become surrounded by filamentous cages formed by intermediate filaments. Coexistence of the forming lipid droplets with the endoplasmic reticulum in the cortical zone of the yolk nucleus and their later investment by intermediate-sized filamentous cages suggest that the yolk nucleus is the birthplace of lipid droplets.

1. Introduction

Animal egg cells usually undergo a considerable and spectacular growth during their formation (oogenesis). This growth results from the accumulation of several different macromolecules and organelles. Some of the macromolecules, like proteins, lipids and carbohydrates, as a rule accumulated and stored in huge amounts in the egg cell cytoplasm (ooplasm), are nutritional reserve materials that are used during embryogenesis as energy resources or substrates for cellular syntheses. Some other, much less abundant, (e.g. different classes of localized ribonucleoproteins (RNPs)), constitute maternally derived developmental information (reviewed in Kloc and Etkin, 2005). Accumulation of reserve substances in the ooplasm requires intense endogenous production and/or active incorporation of molecules from the external environment (blood, hemolymph). The massive and usually rapid character of these processes makes the forming egg cell (oocyte) a good model to study the mechanisms of several essential cellular processes, e.g. endocytosis, synthesis and accumulation of macromolecules etc.

The oocytes often show distinct asymmetry in the distribution of macromolecules and organelles within the ooplasm. For instance, several different subclasses of RNPs are targeted and anchored to discrete ooplasmic localizations. During early embryonic development these RNPs are asymmetrically segregated to the daughter cells as cell fate determinants (reviewed by Kloc et al., 2002; Kloc and Biliński, 2003; Huynh and St Johnston, 2004; King et al., 2005; Kloc and Etkin, 2005; Heasman, 2006; Du et al., 2007). In contrast to ribosomes, great numbers of which are usually more or less evenly distributed within the ooplasm, some other organelles may form assemblages. The earliest reports on such organelle groupings in the oocytes date back to the second half of the nineteenth century. In the pioneering studies on oogenesis, Wittich, Balbiani and others described accumulations of organelles and cytoplasmic inclusions in the oocytes of spiders and myriapods (Wilson, 1904). Originally those prominent aggregations were termed “yolk nuclei”, and then “Balbiani vitelline bodies” or briefly “Balbiani bodies” (for a historical background see: Guraya, 1979). Subsequently, such aggregations have been found in various animal groups (for reviews see: Guraya, 1979; Kloc et al., 2004). Some of them showed many similarities in structure, histochemical composition and behavior, while in some other

* Corresponding author. Tel.: +48 71 375 40 25; fax: +48 71 322 28 17.
E-mail addresses: ijowska@biol.uni.wroc.pl (I. Jędrzejowska), jkubrak@biol.uni.wroc.pl (J. Kubrakiewicz).

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situations they were strikingly diverse. Although many hypotheses were proposed on the role(s) that organelle accumulations might play during oogenesis, their function remained a riddle for decades. Application of contemporary molecular techniques in combination with the ultrastructural analysis in recent years provided important new data on the structure, function and behavior of the Balbiani bodies. These studies were carried out mainly on model organisms, most extensively on the African clawed frog, *Xenopus laevis*. In *Xenopus* oocytes, the Balbiani body is a transient and rather dynamic aggregate of RNA-rich fibro-granular material associated with numerous mitochondria (Heasman et al., 1984; Kloc et al., 2001, 2004). Molecular analyses showed that the Balbiani body contains several different classes of localized RNPs. Based on the data obtained from observations and experiments made on *Xenopus* it is now widely accepted that the Balbiani body represents a transport vehicle for localized RNAs, especially those involved in germ cell fate determination (Kloc and Etkin, 1995; Kloc et al., 1998, 2000; Wilk et al., 2004; reviewed in: Kloc et al., 2004). The functional significance of the close association between the fibro-granular material and mitochondria within the Balbiani body has not been fully elucidated. It was postulated, however, that this association might be a prerequisite for proper selection and inheritance of maternal mitochondria (Marinos and Billett, 1981; Mignotte et al., 1987; Tourte et al., 1981, 1984).

Comprehensive studies of diverse aspects of oogenesis revealed the occurrence of the Balbiani bodies in many animal species even as distantly related as insects (Bradley et al., 2000; Cox and Spradling, 2003; Jaglarz et al., 2003) and mammals (De Smedt et al., 2000; Pepling et al., 2007; Kloc et al., 2008). Among spiders, structures resembling Balbiani bodies were only exceptionally reported (Jędrzejowska and Kubrakiewicz, 2007). Most characteristic for several spider species studied so far are the yolk nuclei, very complex aggregates of various organelles and inclusions usually arranged into orderly concentric zones (Guraya, 1979)[1]. Yolk nuclei not only differ in their composition from Balbiani bodies such as those found in *Xenopus*, but also exhibit a significantly different behavior. Hence, these two structures are clearly discriminated here. Aggregates of organelles with fibro-granular material that appear in the oocytes only transiently (such as those in *Xenopus*) will be referred to as Balbiani bodies, while those with complex and characteristic zonal organization, persisting till the final stages of oogenesis (as found in spider oocytes) will be termed “yolk nuclei” throughout this text.

To date, neither the function of yolk nuclei during oogenesis nor their ultimate fate in the mature egg or embryos have been clearly defined. Cytochemical studies have shown that yolk nuclei in spider oocytes are rich in lipids. Lipids are known to be one of the essential reserve substances accumulated in the growing oocytes. Stored within lipid droplets (LDs), they were considered as energy materials and precursors for membrane synthesis. Recently, lipid accumulation has been additionally linked with several other cellular functions, while LDs appear to be much more dynamic organelles than they were considered in the past (Cermelli et al., 2006; Welte, 2007; Ducharme and Bickel, 2008; Fujimoto et al., 2008; Walther and Farese, 2009). Our knowledge of the ways lipids accumulate in the oocytes is still limited, and comes mainly from studies on a few model insect species (for a review see: Ziegler and Antwerpen, 2006). In spider oocytes, LDs account for a large proportion of the nutritional substances accumulated during oogenesis. Among nutritional materials, LDs are usually first to appear and thus during early oogenesis they become most abundant (Guraya, 1979). Their synthesis or incorporation must then be intense and massive. We do not know whether spider oocytes are able to take up lipids from the external environment (hemolymph), but since it was often reported that LDs appear first in the cortical zones of the yolk nucleus (Guraya, 1979), it was thus tempting to speculate whether the yolk nucleus is a site for LDs formation. By means of precise ultrastructural analysis and specific cytochemical detection of the yolk nucleus components we demonstrate here that it is primarily involved in LD biogenesis. Moreover, we show that LDs arise and mature in association with cisterns of the ER and a well-developed cytoskeletal scaffold formed by condensed filamentous actin and intermediate-sized filaments.

2. Material and methods

For the use of this study, young and adult females of leaf curling sac spiders, *Clubiona* sp. were collected in the field in SW Poland.

2.1. Light and transmission electron microscope

For histological and ultrastructural observations, ovaries were dissected and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH = 7.4) for long periods (usually for a few days). After fixation with glutaraldehyde. The material was repeatedly rinsed with phosphate buffer and for better tissue preservation and contrast was subsequently postfixed in a 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 analyses

2.2.1. Detection of DNA and RNA on semithin sections

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). The material was rinsed with PBS, dehydrated in ethanol series and embedded in acrylic resin Histocryl (Agar, Stanstead, UK). Differential detection of nucleic acids was achieved by staining of semithin Histocryl sections (1.5 μm) sequentially with DAPI (4′,6 diamidino-2 phenylindole dihydrochloridae) (0.2 μg/ml; Sigma Chemical Co., St. Louis, MO, USA) and propidium iodide (0.5 mg/ml; Serva, Heidelberg, Germany). The sections were examined in the Olympus BHS light microscope equipped with an epifluorescence device. Bright red fluorescence of propidium iodide was excited with 546 nm wave (green light), while simultaneous fluorescence of both propidium iodide and DAPI was excited by UV. Blue fluorescent DAPI specifically associates with DNA, while propidium iodide binds both DNA and RNA giving red emission. Used together with DAPI, propidium iodide produced red fluorescence only with RNA-containing structures, while its emission in association with DNA was entirely masked by the much brighter DAPI fluorescence and thus enabled to distinguish DNA from RNA on the same section (for more details see: Tworzydło et al., 2005).

2.2.2. Detection of lipids

For detection of lipids the ovaries were fixed in 4% formaldehyde in PBS. After washing with PBS the ovaries were stained for 30 min

[1] It is worth noting that in some spiders the occurrence of yolk nuclei has not been evidenced (see e.g. Michalik et al., 2005). Why yolk nuclei appear in the oocytes of some species while they do not exist in the others is a question that should be addressed in future comparative investigations.