



VITELLOGENIN TRANSPORT IN THE OVARY OF SOCIAL HYMENOPTERA

José Eduardo Serrão^{1*}, Milton Ronnau², Dihego O. Azevedo³ & Maria do Carmo Q. Fialho⁴

¹Department of General Biology, Federal University of Viçosa, Viçosa, MG, Brazil. ²Department of Biology, Federal University of Paraná, Palotina, PR, Brazil. ³Federal Institute of Espírito Santo, Ibatiba, ES, Brazil. ⁴Department of Morphology, Federal University of Amazonas, Manaus, AM, Brazil.

*Author for correspondence: jeserrao@ufv.br



Laboratory of Cell Ultrastructure

Introduction

Vitellogenesis involves the synthesis of lipoglycoproteins named vitellogenins in the fat body, which are the main yolk compound in the oocytes. The vitellogenin is synthesized on a large scale in the fat body, released into the hemolymph and captured by the developing oocytes, where vitellogenin is converted to vitellin to be used during embryogenesis. However, vitellogenin need to pass the follicular epithelium that surrounds the oocyte, but the mechanisms by which these protein cross the follicle cells barrier on social Hymenoptera remain unknown.

Objective

This study identified the route of vitellogenin transport from the hemolymph to the oocyte in representatives of ants, bees and wasps.

Materials and Methods

Queens of bees (*Apis mellifera* and *Melipona quadrifasciata*), ant (*Pachycondyla curvinodis*) and wasp (*Mischocyttarus cassununga*) were obtained from nests in Viçosa, state of Minas Gerais, Brazil. After dissection, ovaries were transferred to specific fixative solutions.

For immunofluorescence of cytoskeleton and vitellogenin, previtellogenic and vitellogenic ovarioles were transferred to Zamboni's fixative solution for 15 min, then washed in phosphate-buffered saline (PBS) and incubated for 4 h in a 1:100 dilution of phalloidin rhodamine conjugated (cytoskeleton) or anti-vitellogenin followed for incubation with anti-IgG FITC conjugate. After washing samples were mounted in 50% sucrose in PBS and analyzed in a Zeiss LSM Confocal Laser fluorescence microscope.

For ultrastructural analyses, previtellogenic and vitellogenic ovarioles were transferred to 2.5% glutaraldehyde in 0.2M sodium cacodylate, post-fixed in 1% osmium tetroxide, dehydrated 70% ethanol and embedded LR-White resin. Ultra-thin sections were stained with lead citrate and uranyl acetate and analyzed in a Zeiss EM 109 transmission electron microscope

For immunolocalization of vitellogenin, previtellogenic and vitellogenic ovarioles were transferred to 0.5% glutaraldehyde, 1% paraformaldehyde in 0.2 M sodium cacodylate buffer for 30 min. Then samples were dehydrated in 70% ethanol and embedded in LR-White resin. Ultra-thin sections were incubated for 1 h in anti-vitellogenin antibody, washed in PBS and incubated in anti-IgG gold conjugated (10 nm particles). After washing, samples were stained with lead citrate and uranyl acetate and analyzed in a Zeiss EM 109 transmission electron microscope.

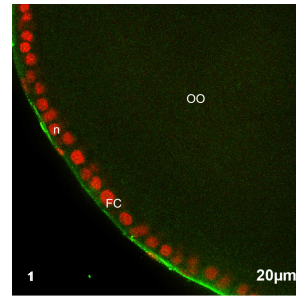
Results

The immunolocalization of vitellogenin (Fig. 1) associated with the actin filaments pattern (Figs. 2A, 2B) in the follicular cells showed that vitellogenin was found in the cytoplasm of follicular cells, suggesting a trans-cellular route although a disorganization of actin filaments occur.

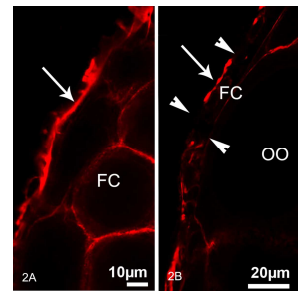
Moreover ultrastructural analysis of follicular cells showed enlarged intercellular spaces in 2/3 of basal cell region (Fig. 3), but at 1/3 apical follicle cells were juxtaposed with the presence of extensive septate junctions, suggesting both trans and paracellular routes (Fig. 4).

The immunolocalization of the vitellogenin showed the presence of this protein within a reticular system in the cytoplasm of follicular cells (Figs. 4 and 5).

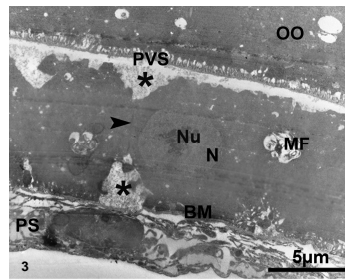
All studied species showed similar features in relation to vitellogenin transport across the follicular cells.



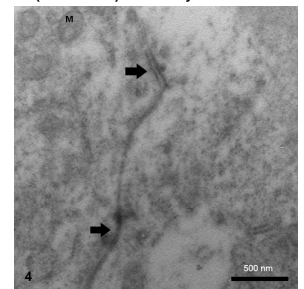
Pachycondyla curvinodis - Immunofluorescence showing vitellogenin within oocyte (OO) and follicular cells (FC). n - nucleus (propidium iodide).



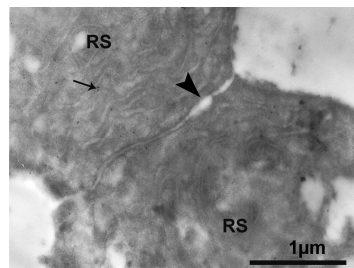
Pachycondyla curvinodis - Actin filaments (arrows) in follicular cells (FC) of previtellogenic (A) and vitellogenic ovaries (B). Note lacking of actin (arrowheads). OO - oocyte.



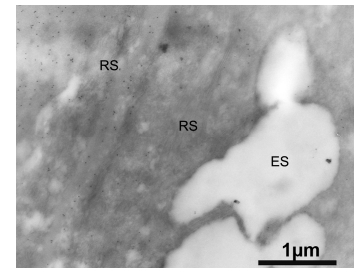
Mischocyttarus cassununga - Micrograph of vitellogenic follicle showing enlarged intercellular space (*) in the base and apex of follicular cell and narrow intercellular space (arrowhead) in the middle region. MF - myelin figure, N - nucleus, NU - nucleolus, OO - oocyte, PS - peritoneal sheath, PVS - previtellic space.



Melipona quadrifasciata - Micrograph of two follicular cells of the vitellogenic follicle with narrow intercellular space containing septate junctions (arrows). M - mitochondria.



Apis mellifera - Immunogold showing vitellogenin (arrow) in the cytoplasm and reticular system (RS) of follicular cell. Note absence of vitellogenin in the extracellular space (arrowhead).



Immunogold showing vitellogenin (dots) in the cytoplasm and reticular system (RS) of follicular cell. Note absence of vitellogenin in the extracellular space (ES).

Discussion and Conclusion

In our hypothetical model, during vitellogenesis, changes in the actin filaments result in the separation of follicular cells with the formation of enlarged intercellular spaces in the basal portion and apical regions of adjacent cells, but with the maintenance of narrow intercellular spaces in the median region owing to septate junctions that are impermeable to vitellogenin. Thus, vitellogenin is transported by a paracellular route from the hemocoel to the median portion of the follicular epithelium, followed by a transcellular route to the dilated intercellular space in the apical region, as a shortcut to the rapid transport of large amounts of vitellogenin. In conclusion, the transport of vitellogenin in the ovaries of social Hymenoptera begins by a paracellular route until the mid-apical portion of the follicle epithelium, and then follows a transcellular route to the perivitelline space.