

ATG6 is required for the endocytosis of yolk proteins during oogenesis of the insect vector *Rhodnius prolixus*

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In insects, all nutrients necessary for the embryo to develop are delivered to the oocytes during oogenesis by receptor-mediated endocytosis, being stored in organelles from endosomal origin called yolk-granules. The contents of the yolk-granules are degraded in a regulated manner during early embryogenesis, and its products are used by the embryo cells for synthesis of fundamental macromolecules. Autophagy is a regulated mechanism that leads to the degradation of intracellular organelles and complexes. The convergence of endocytosis and autophagy has been described, but the mechanisms involved in this crosstalk remain poorly understood. ATG6 is an autophagy-related gene that functions as part of a Class III phosphatidylinositol-3-kinase complex known to regulate the formation of the autophagosome in autophagy as well as the endosome biogenesis and membrane transport. Here, we investigate the role of the protein ATG6 in the endocytosis of yolk proteins in the insect vector *R. prolixus*. The sequence of RpATG6 was identified and analyzed *in silico*. Using qPCR, we found that RpATG6 mRNA is expressed throughout oogenesis with higher expression levels (up to 50%) in pre-vitellogenic and vitellogenic oocytes when compared to full grown oocytes. RpATG6 silencing using RNAi resulted in the accumulation of yolk proteins in the insect hemolymph, suggesting a deficiency in the ability of the oocytes to uptake those macromolecules. Accordingly, we found a variety abnormal morphologies in the oocytes of silenced females. 45 % of the oocytes have a white color (as opposed to the pink oocytes produced in control animals), are smaller and/or collapsed. Silenced females have an overall 28 % increase in oviposition and 60% embryo inviability. Next we intend to test if the silencing of RpATG6 affects the uptake of the main yolk protein (VT), by injecting vitellogenic females with fluorescently-labeled VT and looking at the vitellogenic oocytes using laser-scanning confocal microscopy.

Key words: Autophagy; Endocytosis; Atg6; *Rhodnius prolixus*.