RECEPTOR-MEDIATED ENDOCYTOSIS OF INSECT YOLK PROTEINS

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INTRODUCTION

The frontispiece of William Harvey's "De Generatione Animalium" (Concerning the Generation of Animals) published in 1651 shows the hand of Jove holding an egg, or rather an eggshell, out of which have emerged a wide variety of animals: a child, a dolphin, a spider and so on. Worked into the design is the motto "Ex ovo omnia"--everything comes from an egg. The egg is surely one of nature's most remarkable and versatile inventions. It is a compact self-contained capsule containing everything necessary for the creation of a new life--be it a slug, a worm, a moth, a shark, an ostrich or a turtle.

The major source of nutrients for the developing embryo of all oviparous animals is the egg yolk which constitutes the non-organellar content of the oocyte. The oocyte's dramatic growth during the final stages of maturation is a result of massive deposition of yolk proteins, a process called vitellogenesis. The yolk proteins are in most animal species lipo-phospho-glyco-proteins. They are stored during oogenesis and constitute a depot of amino acids, lipid, carbohydrate and other compounds during embryogenesis. In addition considerable amounts of lipids and glycogen can be accumulated in eggs.

The process of oogenesis has been described in detail for a very large number of animals including insects. However, the molecular basis of this process, the question of "how egg yolk is made" is not well understood. As early as in 1964 Roth and Porter had already demonstrated, in electron microscopic studies on the uptake of yolk proteins by mosquito oocytes, that coated pits pinch off from the oocyte cell surface and form coated vesicles that transport extracellular fluid into the cell. Recently extensive studies on the LDL receptor system showed directly that receptor molecules clustering in coated pits is the essential event in this kind of endocytosis and thus established receptor-mediated endocytosis as a distinct mechanism for the transport of macromolecules across the plasma membrane. Subsequently many additional systems of receptor-mediated endocytosis have been defined and variations of the overall pathway have been described.

Recently, a series of studies have begun in several insect species aimed at gaining a better understanding of the cellular and molecular mechanisms involved in insect oogenesis and yolk formation. I would like to summarize this recent work with some emphasis on our studies in the locust, Locusta migratoria

INSECT VITELLOGENESIS

In insects the process of vitellogenesis includes the production of the yolk protein precursor(s), their transport to the ovaries and their sequestration by developing oocytes. Insect ovaries consist of ovarioles containing oocytes surrounded by a layer of follicle cells, and in some ovary types nurse cells are present. In L. migratoria, as well as in cockroaches and some lepidopteran species, the ovaries consist of panoistic ovarioles. In a panoistic ovariole, oocytes surrounded by a layer of follicle cells are lined up like pearls of a chain. In locusts each ovariole matures a single oocyte at a time. The penultimate oocyte
remains at the stage of previtellogenesis until the terminal oocyte is chorionated. In locusts and cockroaches, for example, all ovarioles synchronously mature one egg each within one week. After oviposition of this batch of eggs the next oogenic cycle is initiated.

Locust eggs contain one predominant female specific yolk protein called vitellin. Its precursor in the haemolymph, vitellogenin (Vg), is synthesized as a phospho-lipo-glyco-protein in the fatbody, secreted into the haemolymph and sequestered by maturing oocytes. The Vg of *L. migratoria* has a MW of 560 kDa and contains about 8% lipids and about 13% carbohydrates. It is reassembled from two primary translation products of 225 and 235 kDa, respectively, and by treatment with SDS it can be cleaved to yield 5 subunits (Chinzei et. al., 1981). Vitellin and its precursor Vg are immunologically and electrophoretically identical. However, during uptake Vg is processed to vitellin which is less well incorporated by developing oocytes in re-uptake experiments and has an enhanced capability to aggregate (Roehrkasten and Ferenz, 1985). This is the situation we find in many insect species (Engelmann, 1979; Hagedorn and Kunkel, 1979; Kunkel and Nordin, 1985). However, in several lepidopteran species, for example, formation of protein yolk seems to be more complicated. Several proteins other than vitellogenin are deposited in substantial concentrations. Some of them are derived from the hemolymph and some from follicular epithelium (Kulakosky and Telfer, 1989). Some are not female-specific, such as lipophorin, and their role in embryonic development is unknown.

It is well-documented that in locusts Vg synthesis in the fat body is controlled by juvenile hormone (Engelmann, 1979). Juvenile hormone biosynthesis and its cyclic nature during oogenesis seem to be regulated by factors from the brain (Feyereisen, 1985) including an allatotropin in locusts, or an allatostatin in cockroaches. Additionally, factors from the ovary may also be involved in the control of juvenile hormone biosynthesis (Aden and Ferenz, unpublished data).

**PATHWAY OF YOLK PROTEIN ENDOCYTOSIS**

Vg is extraovarially synthesized by the fat body and then transported to the maturing oocytes and sequestered by them. To reach the oocyte surface Vg has to pass the basal membrane of the ovariole and to move through the interfollicular spaces between the follicle cells surrounding the oocytes. The yolk protein uptake is accomplished by endocytosis. At the oocyte surface numerous coated pits can be observed, which protrude toward the cortical ooplasm and which are pinched off as coated vesicles. Coated pits and coated vesicles consist of 3 layers: a hexagonal or pentagonal lattice meshwork on the cytoplasmatic side, the unit membrane and a coat of the yolk protein's precursor to be incorporated on the luminal side. The lattice forms a basket around the pits or vesicles of the protein clathrin. Recently, we have been able to isolate coated vesicles from maturing locust oocytes. Like coated vesicles from pig brain and chicken oocytes they have a basket structure containing clathrin as the major protein component (Roehrkasten and Ferenz, 1987b). Roth and Porter (1964) first demonstrated that Vg of *Aedes aegypti* is sequestered by developing oocytes from the maternal haemolymph by adsorptive endocytosis. Later Roth and coworkers (Roth et. al., 1976; Yusko, et. al., 1981) gave a detailed description of this process in chicken oocytes.

The uptake of insect Vg has all the characteristics of a phenomenon called receptor-mediated endocytosis, in which a membrane enclosed receptor molecule specifically binds Vg as its ligand which is then brought into the cell via the coated pits. The receptor-ligand complexes seem to concentrate in coated pits which pinch off from the oocyte membrane to form coated vesicles. The role of the clathrin lattice may be either to anchor the receptor-ligand complex to specialized membrane regions or to help to generate the force to transform a pit into a vesicle. During their movement they seem to lose their clathrin latticework and then fuse to form large yolk spheres. Some parts of the coated vesicles, clathrin lattice, membrane material and the receptor--are probably recycled, since no such protein occurs in a 1:1 ratio with Vg in the oocytes or is synthesized by the maturing oocyte. In addition, there may also exist a very large intracellular pool of receptors. At present many of these steps are not understood, but ongoing research forms a promising and important starting point to explain the process of receptor-mediated Vg endocytosis in insect oocytes.