A diversity of yolk protein dynamics and function

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Abstract

In oviparous (egg-laying) organisms, the accumulation of yolk proteins into the yolk platelets of oocytes is vital for supporting the nutrient demands of embryogenesis. This review will focus on the process through which yolk reserves are stored in the growing oocytes, making up the yolk of the mature eggs. We will discuss yolk granule protein synthesis, targeting, and packaging as well as yolk platelet function(s) during embryogenesis. We begin by discussing what is known about yolk platelet biology in diverse organisms including vertebrates and insects and then compare these observations to what has recently been learned about yolk protein biology in the sea urchin.

Vitellogenesis and oocyte maturation

The majority of animals are oviparous (egg-laying). In these organisms, the accumulation of yolk proteins into the yolk granules of oocytes is of vital importance because it is crucial for oocyte growth maturation; a prerequisite to reproduction. Yolk is not
a single substance, but is a mixture of proteins, lipids, and carbohydrates that are used for nutrition during embryonic development. In oviparous animals, development occurs entirely within the confines of an egg laid in an external environment. Thus, the nutrients provided by the egg contents will enable the production of viable offspring by providing sustenance for the growing embryos until they are capable of feeding on their own (reviewed in De Loof et al., 1998).

Historically, vitellogenesis has been defined as the process through which food reserves are stored in the growing oocytes, making up the yolk of the mature egg. In oocytes of different species, the yolk storage compartments are quite different in appearance due to the composition of yolk, oocyte size, and reproduction strategy of the animal. The majority of oocyte yolk proteins are imported into the oocyte and the major sites for synthesis are distant tissues such as the liver in vertebrates and the fat body in insects (Table 1). In some species, the follicle cells also synthesize a relatively small amount of yolk. Yolk synthesis begins when females become sexually mature and oocyte growth is activated. The sexually mature female then synthesizes and secretes gonadotrophic hormones to stimulate oocytes to grow. Subsequently, cell layers surrounding the oocytes secrete large amounts of sex steroid hormones, namely estrogens in vertebrates and ecdysone in insects. These hormones signal the development of the oocytes and supporting follicles. The liver of egg-laying vertebrates and the fat body of insects respond to these same hormonal cues and start to synthesize large amounts of vitellogenins that are secreted into the circulation. Vitellogenins then bind to specific receptors on the surface of the oocyte and are internalized by endocytosis (Byrne, 1989; Valle, 1993; Sapperston and Raikhel, 1998a,b).

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<th>protein name</th>
<th>organism</th>
<th>size</th>
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<tr>
<td>vitellogenin (Vg)</td>
<td>frog, chicken, fish, nematode, some insects</td>
<td>400-500 kDa</td>
<td>yes, females only</td>
<td>vertebrate: liver, nematode: intestine, insects: fat body</td>
<td>large precursor is cleaved into smaller units, prior to yolk packaging</td>
<td>LDL-like Lp in vertebrates: 95-115 kDa, insects: 180-240 kDa</td>
<td>nutrition &amp; carrier protein for hormones and micronutrients</td>
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<td>yolk proteins (YP)</td>
<td>Dipteran insects (e.g. Drosophila)</td>
<td>46-59 kDa</td>
<td>yes, females only</td>
<td>fat body and ovarian follicle cells</td>
<td>no difference between hemolymph and yolk form</td>
<td>LDL-like 210 kDa</td>
<td>nutrition &amp; carrier protein for ecdysone</td>
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<td>major yolk protein (MYP)</td>
<td>echinoderms (e.g. sea urchin)</td>
<td>180-200 kDa</td>
<td>no</td>
<td>intestine and nutritive phagocytes of ovary and testis</td>
<td>slight difference between coelomic fluid and yolk form</td>
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**Yolk storage proteins**

Animals contain two main classes of storage proteins. One group is called vitellogenin (Vg) and is found in frog, chicken, nematode, fish, and some insects such as the mosquito. The second group is referred to as yolk proteins (YPs) and is found in dipteran insects such as fruitfly, housefly, and the fleshfly. Both classes of proteins are female-specific proteins that are selectively endocytosed by the oocyte and packaged into yolk granules. Although these two classes of proteins do not share sequence
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similarity, they do serve similar functions by provisioning eggs with the nutritional reserves necessary for embryogenesis (Bownes, 1992).

Vitellogenins are similar between phyla

Vgs are large (200-700 kDa) phosphoglycerolipoproteins that serve as major constituents of the egg yolk of almost all oviparous animals. Vg synthesis occurs at extra-ovarian sites including the liver of chickens, frogs, and fish, the fat body in insects, and the intestine in nematodes (Wallace, 1978; Kimble and Sharrock, 1983; Sharrock, 1984; Byrne, 1989). This protein is secreted into the circulatory system and subsequently internalized by developing oocytes through receptor-mediated endocytosis.

Vg is usually post-translationally processed in many ways that differs among phyla. Most insect Vgs are cleaved into large and small subunits prior to secretion into the hemolymph (Dhadiialla and Raikhel, 1990; Dhadiialla et al., 1992), while vertebrate Vg cleavage occurs after internalization by the oocyte (Table 1). One of the vertebrate cleavage products is lipovitellin that is comprised of a heavy chain of 120 kDa and a light chain of 30 kDa. These polypeptides are the major lipid-protein complex found in the yolk and can be associated with varying amounts of noncovalently bound lipids (about 16% w/w lipid). Another proteolytic product of vertebrate vitellogenin is phosvitin (10-35 kDa) that has a high serine content; up to 50% of the protein in some vertebrates (reviewed in Anderson et al., 1998). The role of the serine rich domains is unknown and not all insect Vgs even contain these domains. It is speculated that they serve as substrates for kinases since vertebrate phosvitins are heavily phosphorylated (Byrne et al., 1989). Mosquito Vg is also heavily phosphorylated although the exact phosphorylation sites have not been identified (Dhadiialla and Raikhel, 1990). This phosphorylation may play a role in receptor recognition since dephosphorylation of mosquito and chicken Vg reduces their uptake by oocytes (Dhadiialla et al., 1992; Miller et al., 1982). Alternative explanations of these phosho-serine traits include making a strong negative charge, which may make Vg more soluble (Gerber-Huber et al., 1987) or to allow for chelation of essential metal ions (Nardelli et al., 1987; Taborsky, 1991).

Although the primary function of Vg is to serve as a pool of amino acids for the developing embryo, some constraints on its structure are likely imposed by the requirement for receptor recognition and by its role as a transporter of other molecules. The sequences of vitellogenins of chicken, Xenopus laevis, Caenorhabditis elegans, and a number of insects have been reported (Protter et al., 1982; Gerber-Huber et al., 1987; van het Schip et al., 1987; Wahl et al., 1988; Romans et al., 1995) and protein alignments demonstrate their homology (Nardelli et al., 1987; Byrne et al., 1989; Spieth et al., 1991). Chen et al. (1997) constructed an alignment of multiple sequences and pairwise comparisons of subdomains among members of the same vitellogenin family showed a 25-36% identity between insect members, 35-52% identity between vertebrates, and 37% between different nematodes. Comparisons between phyla show 20-33% identity between vertebrates and nematodes, 18-26% identity between insect and vertebrate, and 18-22% between insect and nematode. Despite the high divergence between phyla, certain positions of amino acid residues are conserved and suggest that yolk precursors of different phyla are members of the same protein family (Chen et al., 1997).
Vg also serves as a transport vehicle carrying covalently linked carbohydrates, phosphates, sulfates, and noncovalently bound lipids, hormones, vitamins, and metals (reviewed in Bownes, 1992). Thus, in addition to the amino acid stockpile it provides, Vgs are responsible for the major delivery of many materials necessary for the metabolic and regulatory requirements of an embryo. In *Xenopus laevis* for example, zinc is derived from the maternal liver as part of a metallo-complex with vitellogenin that is delivered to the oocyte (Montzori et al., 1995). Once these oocytes are fully mature they contain a zinc concentration of about 1mM with 90% of the zinc stored in yolk platelets and 10% in the cytoplasm. The cytosolic fraction is transferred to newly formed metalloproteins involved in early embryonic development while the yolk platelet stores are used later during metamorphosis (Falchuk, 1998). The importance of zinc provisioning is very apparent in *Xenopus*, since embryos incubated in the presence of a zinc chelator do not develop normally (Jornvall et al., 1993). An average of 74% of the embryos hatch, but are malformed and exhibit the classic teratology of zinc deficiency including stunted size, lack of head structures, and missing somites and hearts. The molecular basis of these effects has recently been studied and it appears that the prime function of intracellular zinc is its role in enabling transcription factor function, thereby controlling both qualitative and quantitative aspects of gene-specific transcription (reviewed in Falchuk, 1998). For example, in *Xenopus* the transcription factor TFIIIA is a zinc metalloprotein that binds specifically to the SS RNA gene and activates its transcription. If TFIIIA is present in its apoform it is no longer functional and SS RNA is not formed (Hanas et al., 1983, Miller et al., 1985).

**Dipteran yolk proteins are distinct from vitellogenins**

The yolk proteins (YPs) of dipteran insects, including *Drosophila melanogaster*, are often referred to as vitellogenins even though they are distinct in terms of amino acid sequence, size, processing, and site of synthesis. (Table 1; reviewed in Bownes, 1992).

The YPs form a small gene family of three genes, each encoding a single polypeptide. These three proteins share a high degree of similarity to one another (43%) and an unexpected sequence similarity to triacylglycerol lipases and lipoprotein lipases of vertebrates, including rat, pig, and human (Garabedian, 1987). Unlike vitellogenins, they are not made as large precursors that are processed before storage into yolk. Instead, they are smaller in size (46-50 kDa) with the circulating hemolymph form being similar to the stored yolk platelet form (Bownes and Hames, 1977). In addition to being synthesized in the fat body and secreted into circulation, these proteins are also synthesized and secreted by the somatic follicle cells that surround each developing oocyte. YP deposition into oocytes is essential for development of the embryo as seen by specific mutations that reduce the number of yp genes. Ultimately this condition leads to female sterility by reducing both the number of eggs laid and the amount of yolk deposition which severely compromises embryo viability (Butterworth et al., 1992).

Bownes et al. (1988) showed that even though the YPs of *Drosophila* have sequence similarity to lipases, they do not have lipase activity. They therefore hypothesized that the structural similarity between YPs and lipases might instead reflect domains involved in substrate binding. Indeed, they found that the YPs bind apolar conjugates of ecdysteroids, which are molecules similar to triacylglycerol. Based on the spatial and temporal dynamics of YPs, Bownes et al. (1988) proposed that YP degradation leads to
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Additional yolk proteins

Although Vg and YP are abundant proteins in yolk granules, other proteins are also packaged in these storage vesicles. In insects, a number of hemolymph proteins other than Vg are endocytosed by the oocyte and stored within the yolk granules during vitellogenesis. A vitellogenic cathepsin B (VCB) thiol protease has been identified in the yellow fever mosquito, Aedes aegypti. This protein is synthesized by the fat body of female mosquitoes during vitellogenesis in response to blood feeding and secreted as a latent proenzyme in a sex-, stage-, and tissue-specific manner. During this time the oocyte size increases more than 300 times within 36 hours. Like Vg, VCB is internalized by oocytes and packaged into yolk platelets. At the onset of embryogenesis, VCB enzymatic activity is activated and it degrades Vg whereby the nutritional provision of yolk becomes available (Cho et al., 1999).

Oocyte development in insects also involves the accumulation of large amounts of lipid, most of which is evoattary in origin and is delivered by lipophorin (Lp). Lp is the major lipoprotein complex in both male and female insect hemolymph where it shuttles lipid from the site of absorption in the midgut to either storage in the fat body or metabolically active tissues such as the flight muscle (Canavoso et al., 2001). Lps consist of three constituent apolipoproteins that are synthesized in the fat body. Apolipoprotein I (245 kDa) and apolipoprotein II (78 kDa) are synthesized as a single precursor protein that is proteolytically cleaved into two subunits while apolipoprotein III (18.4 kDa) is a separate gene product. The Lp complex consists of a phospholipid protein surface and a neutral lipid core that varies in composition. Based on their buoyant densities these complexes have been classified as low-density lipoproteins (LDLp), high-density lipoproteins (HDLp), and very-high-density lipoproteins (VHDLp), similar to the vertebrate classification even though vertebrate lipoprotein complexes vary in size and composition when compared to insect (Canavoso et al., 2001).

Lp plays a role in vitellogenesis of Lepidoptera (scalcy-winged insects including butterflies and moths); it shuttles lipid to the ovaries and in some instances becomes one of the major constituents of the egg yolk proteins (Telfer et al., 1991). In Manduca sexta (tobacco hornworm) up to 40% of the dry weight of the mature egg is lipid (Kawooya and Law, 1988). Biosynthesis of lipid in the egg accounts for about 1% of the lipid and 5% is delivered by the yolk protein vitellogenin (Kawooya and Law, 1988). LDLp and HDLp deliver the rest of the lipid (94%) to the oocyte (Kawooya and Law, 1988; van Antwerpen, 1998).

Yolk endocytosis is a conserved mechanism

Receptor-mediated endocytosis is an essential process for eukaryotic cellular functions including nutrient uptake and recycling of membranes and membrane proteins (Mukherjee et al., 1997). The process of yolk inception and endocytic trafficking proceeds through a pathway that is very similar to those of low density lipoproteins (LDL) and LDL receptors in somatic cells (Schneider, 1996). This interaction is critical for import of cholesterol, an essential component of cell membranes.
The receptor-mediated endocytosis of yolk proteins assures selective, efficient, and accumulative uptake of yolk components. The massive inception of yolk into the oocyte during vitellogenesis requires "heavy" recruitment of the endocytic machinery. Interestingly, oocytes are the cells where clathrin coated pits and vesicles were first observed and morphologically described (Fig. 1; Roth and Porter, 1964). Similar morphological descriptions of this process have been made in a wide range of oviparous species including birds, frogs, fish, and insects (reviewed in Raikhel and Dhadiwalla, 1992).

Figure 1. Surface of the mosquito oocyte during vitellogenesis. This schematic drawing interprets the micrographs examined by Roth and Porter (1964). It depicts the surface of the mosquito oocyte during the period of active pinocytosis and the formation of yolk granules. (1) yolk proteins bind receptors at the surface of the oocyte (2-3) the receptor-ligand complex is internalized into an endosome via clathrin coated pits (4) clathrin dissociates from the endosome (5-7) the endosome fuses with other dense spheres forming transitional yolk platelets (8) together these coalesce and form the large crystalline yolk bodies. cv = coated vesicle; er = endoplasmic reticulum; fc = follicle cell; ld = lipid droplet; mv = microvilli; Y = yolk (From Roth and Porter, 1964).

One of the key components in the uptake of yolk proteins is the receptor. Vitellogenin receptors from birds, insects, fish, and frogs have been identified, and belong to the LDL receptor superfamily (reviewed by Schneider, 1996; Sappington and Raikhel, 1998a). Despite the fact that YPs of dipteran insects are distinct from vitellogenins, the Drosophila yolk-protein receptor, Yolkless (Yl), is also a member of the LDL receptor family (Schonbaum et al., 1995). Females mutant for Yl are sterile and fail to accumulate yolk in oocytes (Waring et al., 1983; Perrimon et al., 1986). In addition, the ovarian lipophorin receptor (LpR) in Aedes aegypti has been identified and also belongs to the LDL receptor superfamily (Fig 2). These findings suggest that the evolution of yolk proteins and yolk-protein receptors has occurred in concert and in parallel with that of similar ligand-receptor systems important for the normal physiology of animals that do not rely on oviposition.

Insect Vg receptors (VgR) and ovarian lipoprotein receptors are large (180-214 kDa) membrane bound proteins, roughly twice the size of vertebrate VgRs (95-115 kDa) (Sappington and Raikhel, 1998a,b). Yl is similar in size (210 kDa) to the insect VgRs
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and like other members of the LDLR family is characterized by a highly conserved arrangement of modular elements (Fig. 2) including: complement-like ligand binding repeats and three types of epidermal growth factor repeats, one of which is characterized by a recurrent ‘YWTD’ sequence. All of these repeats are believed to be involved in the protein-protein interactions necessary for ligand-receptor binding.

The mechanisms of general receptor-mediated endocytosis have been extensively analyzed (Fig. 3; Goldstein et al., 1979; Goldstein et al., 1985; Robinson et al., 1994; Schwartz, 1995). The ligand-receptor complex is endocytosed into an early endosome concomitant with a drop in pH that dissociates these two proteins from one another. The receptor recycles back to the cell surface for another round of endocytosis while the ligand is destined for the lysosome. In this low pH environment, it is hydrolyzed by lysosomal enzymes with the release of its constituents into the cytosol. An example of this process is the release of cholesterol from LDL (Fig. 3) or the release of iron from transferrin.

The initial steps in the yolk uptake pathway are similar to those described for general receptor-mediated endocytosis (Goldstein et al., 1985; Mukherjee et al., 1997). Vitellogenins and YPs bind to their cognate receptors on the surface of the oocyte and the complex is taken up through clathrin-coated pits. These pits initially form vesiculotubular structures that pinch off and fuse to form the primary yolk bodies. In contrast to general endocytic pathways where the internalized ligands are fated for degradation in lysosomes, a specialized mechanism has evolved in oocytes so that yolk proteins are stored in yolk granules for use later in embryogenesis. In order to accomplish this timed release mechanism, yolk granules are packaged with an initial relatively high pH. During embryogenesis, the pH of the yolk granule drops to levels

Yolk protein dynamics

- complement-like ligand binding repeats
- EGF precursor homology domain
- EGF precursor homology domain
- EGF precursor homology domain (spacer region, YWTD)

Figure 2. Schematic representation of the LDLR superfamily proteins.
The domains for comparison are: the complement-like ligand binding repeats with six cysteines each; EGF precursor homology repeats of two subtypes, termed B1 and B2 that also contain six cysteines each; and the YWTD repeats of the EGF precursor homology domain. LDLR represents the mammalian LDLRs; VLDLR represents the chicken vitellogenin receptor, Yl is the yolk protein receptor of Drosophila, and LRP represents the LDLR-related proteins from chicken and humans. (Modified from Schneider, 1996).
Figure 3. Uptake of cholesterol by receptor-mediated endocytosis. The binding of LDL to an LDL receptor initiates endocytosis of this complex into the cell within an endosome. The receptor is recycled back to the cell surface prior and the endosome fuses with the lysosome. The lysosome contains enzymes that hydrolyze LDL releasing cholesterol, fatty acids, and amino acids into the cytosol. (From Lehninger et al., 1993).

more typical of lysosomes, initiating its proteolytic capacity for constituent hydrolysis (reviewed in Fagotto, 1995).

Vitellogenesis in sea urchin

Although yolk is generally known to provide a store of nutrients and energy, its function in sea urchin embryos remains unclear. This uncertainty stems from the observation that the yolk platelets, though similar morphologically to those found in other animals, are not utilized during early development.

In sea urchins, the vitellogenic phase of oogenesis is also characterized by yolk deposition. Sea urchin ovaries are comprised of five sac-like organs located within the perivisceral coelomic cavity (Fig. 4). They are loosely connected to the test, that forms the hard shell of the sea urchin's body, and open to the exterior of the body by five gonopore openings located opposite the mouth. The ovaries are directly in contact with the circulating coelomic fluid and all major organ systems within each animal.
Yolk protein dynamics

Figure 4. Sea urchin anatomy. (From Petrunkevitch, 1875).

Each ovary lobe is comprised of a capsule surrounding a germinal epithelium and a lumen that contains oocytes and mature eggs. The capsule of the ovary consists of an outer sac, a layer of connective tissue that is covered externally with a flagellated visceral peritoneum and internally with flagellated myoepithelial cells. The inner sac of the capsule consists of a genital haemal sinus covered externally with myoepithelial cells and internally with the germinal epithelium. The genital haemal sinus is a fluid-filled space that serves to connect the ovarian tissue to the perivisceral coelom allowing for the translocation of nutrients (Walker, 1982; Beijnink et al., 1984).

The oocytes within the germinal epithelium are commonly divided into three classes: primary, growing, and terminal (Cowden, 1962). At the beginning of oogenesis, the oocyte is about 10 μm in diameter. During the vitellogenic phase of oogenesis (about a one month period), the oocytes accumulate yolk proteins and grow to ten times their original size to 100 μm, the same size as a mature egg. The vitellogenic phase of oogenesis is asynchronous so each ovary lobe has hundreds of oocytes at all stages of development (Fig. 5).

The somatic tissue of the germinal epithelium is composed of accessory cells that are morphologically heterogeneous. One class of somatic cells are the nutritive phagocytes (NPs), an integral part of the oocyte-support structure (Fig. 5). These cells provide glycogen and yolk to developing oocytes during the vitellogenic phase of oogenesis (Verhey and Moyer, 1967; Bal, 1970) and at the end of the gametic cycle they recycle this nutrition by phagocitizing atretic oocytes (Holland and Giese, 1965, Masuda and Dan, 1977). The differentiation and growth of NPs are tightly linked to the mechanical stabilization and growth of oocytes from early oogenesis to egg maturation (Walker, 1982). From early histological studies many investigators concluded that materials in the ovarian accessory cells are translocated into the oocyte during vitellogenesis (Holland and Giese, 1965; Chatlynn, 1969). Studies using electron microscopy (Fig. 6) show that NPs surrounding the oocyte contain granular inclusions abundant in glycoprotein particles that play an important role in the supply of nutrient substances for the oocyte’s growth. Early in the oogenic period, the NPs accumulate...
Figure 5. Sea urchin ovary histology. Sections of a *Lystechinus variegatus* ovary counterstained with pararosaniline. (A) 100X (B) 400X C = perivisceral coelom; L = lumen of the gonad; NP = nutritive phagocytes; OC = ovarian capsule; PO = previtellogenic oocytes; VO = vitellogenic oocyte.

Figure 6. The transfer of glycoproteins during sea urchin vitellogenesis. Electron microscope studies on oocyte endocytosis in the sea urchin *Hemicentrotus pulcherimus* show the transfer of glycoproteins from their storage in large mosaic granules inside the nutritive phagocytes to the periphery of the oocyte where they are endocytosed. (A) Surface layer of the ovary; 11750X. (B) Large mosaic granule in the nutritive phagocyte breaking down (arrow) and releasing contents; 9400X. (C) Surface layer of an oocyte showing endocytic pinosomes; 18250X. CG = cortical granule; GV = germinal vesicle; MG = mosaic granule; NP = nutritive phagocyte; Oo = oocyte, P = pinosome (Modified from Tsukahara, 1971).
large stores of yolk, the mass of which depends on the dietary success of the adult. During vitellogenesis, the plasma membrane of the NPs breaks down releasing the glycoprotein particles that are endocytosed at the periphery of the growing oocyte (Takashima and Takashima, 1966; Tsukahara and Sugiyma, 1969; Tsukahara, 1971; Geary, 1978). These vesicular bodies then form the yolk platelets, which are membrane enclosed deposits of electron dense material, 1-1.7 μm in diameter, distributed evenly throughout the cytoplasm (Gross et al., 1960; Monroy and Maggio, 1963). Yolk platelets are present in large amounts and constitute 28-38% of the total egg weight (Harvey, 1932; Costello, 1939).

At the end of oogenesis the ovary is nearly filled with mature eggs, the NPs have decreased considerably in size, there are very few glycoprotein particles in the space among the oocytes and the NPs, and the transferred glycogen particles are progressively broken down into smaller vesicles within the oocyte (Walker, 1982). These oocytes mature into eggs that are stored within the ovary until the animal is stimulated to release them, which could be for days or weeks. The yolk remains intact during this storage period and is not utilized until late in embryogenesis.

Identification of the major yolk protein in sea urchin

The major components of yolk granules were first isolated from eggs as a 22-27 S particle, by sedimentation velocity centrifugation (Malkin et al., 1965; Infante and Nemer, 1968; Ichio et al., 1978). Curiously, this particle did not appear to change in overall mass during development, contrary to what was expected for yolk catabolism. However, Kari and Rottman (1980) examined the constituents of the 22-27 S particle and found that the major protein, a 200 kDa glycoprotein, decreased in both abundance and size during development and disappeared after the late blastula stage. Harrington and Easton (1982) further investigated the identity of this protein by looking at total egg homogenates from three species of sea urchin. Using the PAS (periodic acid-Schiff) staining procedure they found that this 200 kDa glycoprotein accounted for 10-15% of the total egg protein. They characterized this protein by using a sucrose gradient to fractionate the yolk component of egg homogenates and estimated that 50% of the protein in the yolk fraction was the abundant 200 kDa glycoprotein. They therefore designated it the major yolk protein (MYP). Similar to the results obtained by Kari and Rottman (1980), Harrington and Easton (1982) found that this 200 kDa MYP decreased in concentration during embryogenesis, even though the overall mass of the yolk glycoprotein complex remained constant (Malkin et al., 1965).

Biochemical processing of the major yolk protein

To resolve the conundrum of yolk utilization, Kari and Rottman (1985) investigated the yolk glycoprotein complex of the 22-27 S particles in two species of sea urchin (Lytechinus pictus and Strongylocentrotus purpuratus) and focused on their composition during embryogenesis. They found that the total amino acid composition remained the same during early development. They took a closer look at the decrease of the 200 kDa MYP and showed that its disappearance coincided with the appearance of distinct lower molecular weight glycoproteins. In vivo radiolabeling experiments carried out by Arman et al. (1986) showed that the appearance of these new yolk glycoproteins was not the result of de novo protein synthesis and suggested that instead they represented
proteolytic processing of MYP.

Scott and Lennarz (1989) confirmed that the yolk platelet glycoproteins of lower molecular weight were indeed derived from MYP. They looked at the proteolytic processing of MYP using a polyclonal antibody for immunoblot analysis of total proteins over the course of development and showed that MYP was processed into discrete smaller fragments reflecting an overall preservation of total protein mass. The significance of the limited proteolysis and how it related to yolk utilization was unclear, but it does explain how the 200 kDa MYP protein can disappear from the yolk platelet without a concomitant decrease in yolk platelet mass.

Acidification of sea urchin yolk platelets

Schuel et al. (1975) proposed that sea urchin yolk platelets are analogous to lysosome-like storage particles based on their content of lysosomal hydrolases. The hydrolase activity appeared to be optimal at acidic pH (Schuel et al., 1975; Yokota and Nakano, 1985). Yokota and Kato (1988) tested the hydrolysis of MYP under acidic conditions to ascertain if the changes in the polypeptide sizes were pH dependent. Yolk granules isolated from both unfertilized and fertilized eggs of Hemicentrotus pulcherrimus and Anthocidaris crassispina were incubated in ASW buffered at various pH values (4.2-8.0). Under acidic conditions (pH 4.2) the 180 kDa MYP from H. pulcherrimus disappeared by 6 hours and the 114, 94, 72, and 61 kDa proteins appeared. Similar results were seen for the corresponding MYP in A. crassispina (178 kDa). Electron microscopy showed that freshly isolated yolk granules had a membrane-limited vesicular structure in which electron dense subparticles were tightly packed. The density of these subparticles decreased substantially when they were incubated in acidic artificial seawater (pH 4.2) and remained unchanged when the protease inhibitor leupeptin was added in these same acidic conditions. The decrease in density of subparticles in the yolk granules observed with the electron microscope coincided with the processing of the yolk proteins detected by SDS-PAGE. This study suggested that a low pH environment promotes the processing of yolk proteins in sea urchins by a protease dependent method. It seemed unlikely that the protease was newly synthesized during development since yolk granules from eggs contained this protease that could be activated at low pH. It was suggested that a proton pump was activated or translocated to the yolk platelet membrane to regulate these pH associated ion fluxes (Scott and Lennarz, 1989).

The pH regulatory capability of yolk granules appears to be a general phenomenon having been observed in arthropods (Fagotto, 1990), and in the amphibian Xenopus laevis (Fagotto and Maxfield, 1994a, b). The studies in Xenopus have shown that yolk platelets contain a vacular proton pump that regulates both granule acidification and vitellogenin degradation. Protease catalysis of these proteins was suggested, as proteases isolated from yolk are essentially inactive at neutral pH but active at lower pH. In both Xenopus laevis and Aedes aegypti (mosquito) the yolk granule proteases have been identified and classified as acid-labile cysteine proteases (Yoshizaki and Yonezawa, 1998; Cho et al., 1999).

Little is known about the specific protease(s) that cleave yolk proteins in sea urchins. The effect of different protease inhibitors on yolk degradation has been examined and the data at hand is inconclusive. Some groups have reported that MYP processing was mainly due to a cysteine protease (Umezawa, 1982; Yokota and Kato
platelet glycoproteins of lower mass. They looked at the proteolytic
activity of total proteins YP was processed into discrete
protease degradation, so the complexity of proteases in the sea urchin yolk platelet may differ substantially.

Major yolk protein synthesis and storage in the ovary and testis

MYP accumulates in the somatic accessory cells of both gonad types (Harrington and Ozaki, 1986; Ozaki et al., 1986). Unuma et al. (1988) used immunohistochemistry to examine the distribution of this protein in both the ovary and the testis during gametogenesis. Prior to gametogenesis, the follicular lumen of the gonads of both sexes of animal is filled with MYP-laden NPs. During gametogenesis, the NPs regress and the lumen of the gonads fills with mature gametes. In females, the accumulation of MYP in ripe ova is coincident with its depletion in the regressing NPs. In males, regressing NPs show MYP depletion but this is not coincident with its appearance in the developing gametes (Unuma et al., 1988). Unuma et al. (1988) conclude that MYP accumulation in the gonads is similar in both sexes of sea urchin although the site of utilization is different. In females it is transported to oocytes and packaged in eggs to be used during embryogenesis and in males it functions as a nutrient for spermatogenesis.

Since many yolk proteins are imported into growing oocytes, a heterosynthetic source of origin for MYP seemed likely. The coelomic fluid of the sea urchin is analogous to the blood of vertebrates or the hemolymph of insects, so Harrington and Easton (1982) analyzed coelomic fluid from adult sea urchins and identified an abundant glycoprotein at a slightly higher molecular weight than the MYP found in yolk homogenates. To establish if there was any identity between these two proteins they used partial proteolysis using Staphylococcal protease V8 and compared peptide maps. The cleavage pattern was similar for the coelomic fluid protein and the yolk protein suggesting a precursor product relationship. This proposal fit the physiological profile of other previously identified vitellogenins that are cleaved or otherwise modified in the course of secretion, transport, or digestion into growing oocytes. Interestingly, they identified the coelomic precursor to MYP in both sexes of sea urchin. This was very different from any other vitellogenin that was characterized at the time.

Using an immunospecific antibody, Shyu et al. (1987) sought the somatic source of MYP. They examined the in vitro translation of mRNA isolated from various tissues and identified a 155 kDa protein precursor in the intestines and gonads of both sexes of adult sea urchins. Immunoprecipitations from these same tissues with the MYP antibody also detected a 195 kDa form of the protein (similar to that seen in coelomic fluid) that they presumed resulted from posttranslational glycosylation. Arman et al. (1986) tested this possibility by digesting MYP from yolk platelets of Arbacia punctulata (170 kDa) with endoglucosidase H and saw a 35 kDa loss in molecular weight, indicating that MYP is heavily glycosylated. This observation is consistent with the earlier histochemical and cytochemical studies that demonstrated that yolk platelets are the major PAS-staining organelles of sea urchin eggs (Immers, 1960; Takashima, 1971). Shyu et al. (1986) observed a second MYP processing step that results in an apparent shift of the 195 kDa MYP to the 180 kDa mature form found in the eggs. This final step only occurs in the ovary, presumably in the oocytes. Further processing in eggs was apparent as
immunoblot analysis of unfertilized eggs showed a primary immunoreactive band at 180 kDa as well as several distinct lower molecular weight bands that are consistent with the proposed cleavage products reported by Kari and Rottman (1985). In summation, Shyu et al. (1987) concluded that MYP is a vitellogenin, even though it is not sex-specific. They suggested that in addition to being a yolk precursor, MYP may serve as a carrier protein for fatty acids analogous to the role of serum albumin in vertebrates.

**Major yolk protein utilization**

Shyu et al. (1986) were the first to raise MYP-specific antibodies and confirm that the protein is specific to yolk platelets by doing electron microscope immunolocalization on unfertilized eggs (Fig. 7). Scott and Lennarz (1989) extended these studies using an antibody to MYP for electron microscopic immunolocalization of fixed embryos. They saw that at all stages of development up to pluteus larva (four days), MYP is localized exclusively in yolk platelets with no evidence for its presence (translocation) in lysosomes (Armant et al., 1986), at the cell surface (Gratwohl et al., 1991), or elsewhere.

![Figure 7](image-url)  
**Figure 7.** Electron microscope immunolocalization of the major yolk protein (MYP) in unfertilized eggs of the sea urchin Strongylocentrotus purpuratus shows MYP in yolk platelets and not other subcellular organelles. (A) MYP preimmune sera; 40,000X. (B) MYP antiserum; 40,000X. CG = cortical granule; YP = yolk platelet; MITO = mitochondria (Modified from Shyu et al., 1986).


Yolk protein dynamics

This study was extended to an even later time frame with the emergence of improved embryo culturing techniques (Scott et al., 1990a). These investigations established that the yolk platelet and MYP disappeared at day 7 of development. This is interesting since the five-day-old larva is fully developed and has begun feeding on its own and can obtain its own nutrition. Furthermore, they established the resynthesis of MYP in premetamorphosis 30-day-old larvae, which is well before gametogenesis and yolk production begins in the female. The observation that premetamorphosis larva, adult males, and adult females synthesize MYP suggests that this protein is necessary for some other function besides being a precursor to yolk in the adult female (Shyu et al., 1987; Scott et al., 1990a).

Alternate nutritional reserves during sea urchin embryogenesis

Fatty acids are plentiful in the yolk platelets (Ichio et al., 1978) yet they do not appear to provide sustenance for the growing embryo. Armant et al. (1986) focused their attention on other sources of lipid in the embryo, most notably the lipid droplets, and reasoned that they could provide a store of catabolizable organic precursors. Indeed, when the lipid content of yolk platelets was compared with the crude lysate it was apparent that the yolk platelet fraction remained constant while the crude lysate fraction was depleted. Electron microscopic analysis also showed that in contrast to the yolk platelets, lipid droplets were seen in the unfertilized egg (Fig. 8) but were rarely observed at the blastula stage, while yolk platelets remained abundant. These data suggest that the nutritional fuel for embryogenesis may reside outside of yolk platelets.

Figure 8. Yolk platelets and lipid droplets in unfertilized sea urchin eggs. Ultrastructural analysis indicates that in addition to yolk platelets, lipid droplets are commonly seen in unfertilized sea urchin eggs. An egg from Arbacia punctulata is seen here. cg = cortical granule; L = lipid droplet; YP = yolk platelet (Modified from Armant et al., 1986).
The maternal provisioning of yolk platelets and lipid droplets is different between sea urchins with divergent modes of development (Scott et al., 1990b). The ancestral mode of sea urchin development is indirect, through a feeding larva, and these species have small eggs (80-100 μm). The alternative and derived strategy of development is represented by direct developers who produce large eggs (430 μm) and a highly modified lecithotrophic larvae that can successfully develop to juveniles without eating. Byrne et al. (1999) examined direct and indirect developers in the Heliocidaris genus of sea urchin. They determined that the increase in egg size of direct developers is not due to yolk provisioning but rather to a second phase of oogenesis characterized by deposition of additional maternal protein and lipids. The production of these large lipid-rich eggs may reflect the energetic reserves necessary to surpass the feeding pluteus stage of typical developers.

Alternate function(s) for yolk platelets

Armant et al. (1986) suggested that yolk platelets might function in lipogenesis or membrane biogenesis due to the observed vesicular structure of these organelles. To test their hypothesis, they took a lysed yolk protein preparation and assayed for enzyme activity including fatty acyl coenzyme A lyase, glycerol phosphate acyl transferase, choline phosphotransferase, and ethanolamine phosphotransferase. No detectable enzyme activity was observed which led to a conclusion that the yolk platelet was not a site of lipid synthesis. Armant et al. (1986) then suggested that yolk platelets might be used later in development and serve as an energy reserve if food is not available when feeding of the embryo begins. However, this doesn’t seem to be the case either since Scott et al. (1990a) showed that at the feeding pluteus stage, the yolk platelets and MYP disappear regardless of the presence or absence of exogenous nutrition.

Recent studies have shown that yolk platelets can provide membrane in the event of either a plasma membrane or cytoplasmic disruption. Within 5 seconds of shearing off more than 1,000 μm² of plasma membrane, eggs of the sea urchin or starfish repair themselves, and can be fertilized and will divide normally (Terasaki et al., 1997). The egg can also erect a new membranous structure when disruptions are introduced deep into the cytoplasm by the microinjection of sea water (McNeil et al., 2000). The yolk platelets have been shown to contribute to this “new” membrane. Upon exposure to extracellular calcium, yolk platelets undergo homotypic fusion and rapidly erect a membrane barrier (McNeil et al., 2000; reviewed in McNeil and Terasaki, 2001). This capacity for membrane repair is important since fertilized eggs are free-living cells confronted by environmental disruptions.

Molecular analysis of the major yolk protein: A transferrin-like iron binding protein

The assignment of MYP as a vitellogenin was initially based on its abundance in yolk platelets, coelomic fluid, and intestines, but not on any sequence data. Shyu et al. (1986) were the first to present a partial cDNA encoding MYP from an adult S. purpuratus intestinal cDNA library. To accomplish this, they fractionated the intestinal poly(A) RNA on a sucrose gradient and performed in vitro translation to determine which fraction was enriched for MYP. They took this mRNA fraction, synthesized a cDNA library, and screened it with both size-selected and unfractionated cDNAs. A
pig droplets is different between cott et al., 1990b). The ancestral feeding larvae, and these species rived strategy of development is of eggs (430 μm) and a highly velop to juveniles without eating. opers in the Heliocidaris genus of ze of direct developers is not due of oogenesis characterized by e production of these large lipid- y to surpass the feeding pluteus.

might function in lipogenesis or cture of these organelles. To test ration and assayed for enzyme erol phosphate acyl transferase, phostransferase. No detectab n that the yolk platelet was not a sted that yolk platelets might be ve if food is not available when seem to be the case either since tage, the yolk platelets and MYP nus nutrition. rovide membrane in the event of Within 5 seconds of shearing off the sea urchin or starfish repair ally (Terasaki et al., 1997). The disruptions are introduced deep (McNeil et al., 2000). The yolk membrane. Upon exposure to pic fusion and rapidly erect a cNeil and Terasaki, 2001). This ilized eggs are free-living cells.

**n: A transferrin-like iron**

ially based on its abundance in any sequence data. Shyu et al. coding MYP from an adult S. s., they fractionated the intestinal n vitro translation to determine mRNA fraction, synthesized and unfractionated cDNAs. A partial cDNA clone (980 bp) was identified and confirmed to represent MYP by in vitro translation of hybrid selected mRNA. This partial clone was then used for RNA gel blot analysis and a single 5.1 Kb transcript in adult intestine, ovary, and testis was detected. No significant signal was seen in eggs or embryos.

The structure of the MYP cDNA clone isolated by Shyu et al. (1986) was reported the following year when it was used to analyze a S. purpuratus genomic cDNA library (Shyu et al., 1987). The 980 base pair cDNA clone represented the 3’ end of the MYP sequence and consisted of a 240 nucleotide open reading frame followed by a stop codon, a polyadenylation signal, and a short poly(A) sequence. This clone was used for genomic Southern blot analysis that suggested that MYP is a single copy gene, unlike most vitellogenins. Then a genomic library screen was performed and several overlapping clones from a 3.3 Kb of sequence at the 5’-end of the MYP gene were identified (Shyu et al., 1987). The putative transcription start site was determined by primer extension, and a canonical TATA box was present 32 bp upstream of this position. Interestingly, a sequence closely resembling an estrogen-response element was present in the putative promoter, suggesting hormonal control of this gene. Such elements are also present upstream of Xenopus and chicken vitellogenin genes (Jost et al., 1984; Klein-Hitpass et al., 1986; Seiler-Tuyns et al., 1986). Sea urchins and other echinodermals also produce estrogen, which appears to affect oogenesis in starfish (Schoenmakers and and Dieleman, 1981; Schoenmakers and Voogt, 1981).

The actual protein sequence data from these two partial clones (Shyu et al., 1986, 1987) did little to resolve the conundrum regarding this protein’s assignment as a vitellogenin and its perplexing role as a nutrition source during embryogenesis since combined they only represented 13% of the predicted ORF and they did not contain sequence similarity to other reported vitellogenins.

The present study found a partial cDNA clone for MYP in S. purpuratus. This is the first time that a full length sequence has been reported from any echinoderm species and the information provided from the sequence significantly improves our understanding of its biology. We present the primary structure of MYP as predicted from cDNAs of two sea urchin species, Strongylocentrotus purpuratus and Lytechinus variegatus (Fig. 9). The sequence from these two species share identity to one another, but bear no resemblance to other known vitellogenins. Instead, the sequence shares identity to members of the transferrin superfamily of proteins. In vitro iron binding assays, including both 59Fe overlay assays of MYP enriched coelomic fluid and immunoprecipitation of native iron-bound MYP from coelomic fluid, support this classification (Fig. 10). We suggest that one of MYP’s transferrin-like properties is to shuttle iron to developing germ cells. Upon closer examination of the literature we find that this new classification of MYP fits into the established physiological framework of this protein and explains many of the incongruities that were originally manifested with its initial characterization as a vitellogenin. We now propose that MYP functions as an iron transporter that supplies iron to support either gametogenesis, embryogenesis, or both (Table 2). We can not exclude other roles for this protein, but believe iron transport is a major function.

Iron is an essential nutrient required for nearly all living organisms. Ionic iron forms oxides that are insoluble at physiological pH. Thus, in order to absorb and transport iron, most organisms have acquired special cofactors and proteins that are capable of maintaining iron in a water soluble state (Yoshida et al., 1999). Transferrin binds iron.
Figure 9. Sea urchin major yolk protein (MYP) cloning. Shown are the maps of the clones used to determine the full-length open reading frame for the *Strongylocentrotus purpuratus* MYP and the partial open reading frame from the *Lytechinus variegatus* MYP. Included is a scale diagram of the putative protein encoded showing the predicted transferrin-like, iron binding domains. *Shyu et al.,* 1986 (From Brooks and Wessel 2002).

Figure 10. Sea urchin coelomic fluid has iron binding capacity. These slot blots show that $^{59}$Fe binds to human serum transferrin and both female and male adult sea urchin coelomic fluid in a concentration dependent manner. It does not bind to gelatin (top) or other non-specific proteins such as BSA and Blotto (data not shown). (From Brooks and Wessel 2002).
Yolk protein dynamics

Table 2. Comparison of properties of vitellogenin-like and transferrin-like proteins. (From Brooks and Wessel, 2002).

<table>
<thead>
<tr>
<th>Property</th>
<th>transferrin-like</th>
<th>vitellogenin-like</th>
</tr>
</thead>
<tbody>
<tr>
<td>expression is sex-specific</td>
<td>no</td>
<td>yes, only</td>
</tr>
<tr>
<td>expression can be regulated by hormones</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>major site of synthesis: liver, fat body, gut</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>secreted into circulation</td>
<td>yes, &amp;</td>
<td>yes</td>
</tr>
<tr>
<td>delivery to germ cells function</td>
<td>transport &amp; sequester Fe</td>
<td>nutrition</td>
</tr>
</tbody>
</table>

Kurama et al. (1995) have characterized an insect transferrin that is transported into oocytes during the vitellogenic phase of oogenesis. Soon after uptake the iron is delivered to another protein believed to be ferritin. Transferrin accumulates in the eggs and is present during early development. Since activation of the transferrin gene is not apparent during this early development, it is proposed that the maternal store of transferrin is solely responsible for the delivery of iron to newly formed embryonic cells. In contrast to this iron exchange, ovotransferrin in avian eggs retains its iron and constitutes about 10% of the total egg white protein. This iron-withholding sequesters iron away from pathogens and serves as a bacteriostatic function in the egg. In mammalian embryos, the transferrin gene is activated during early development and the embryo is also supplemented with maternal transferrin via the placenta (Morgan, 1974; Huxham and Beck, 1985; de Jong et al., 1990). In sea urchins, we have demonstrated that MYP binds iron in coelomic fluid, is delivered to the oocyte, is being endocytosed during the vitellogenic phase of oogenesis, and packaged into yolk platelets (Brooks and Wessel, 2002). We do not know the fate of the iron ions following this process. Iron may remain associated with MYP or it may be transferred to other metalloproteins of the egg such as ovoperoxidase. This protein is made during oogenesis and is packaged into the cortical granules, released during the cortical reaction at fertilization, and incorporated into and crosslinks the proteins in the fertilization envelope. Ovoperoxidase is a heme-dependent peroxidase that requires iron for its activity (LaFleur et al., 1998). MYP likely transfers iron to the iron storage protein ferritin, and by chance we have isolated a cDNA from an

These slot blots show that $^{59}$Fe binds to hyn coelomic fluid in a concentration n-specific proteins such as BSA and
S. purpuratus ovary library that encodes ferritin (unpublished results). MYP could be delivering iron for storage in the nutritive phagocytes if they are harboring ferritin. There have also been some reports of ferritin in yolk platelets in other animals so this too remains a possibility (von Darl, et al., 1994; Kim et al., 2001).

The most well studied transferrins in vertebrates are serum transferrin and lactoferrin in mammals, and ovotransferrin in birds (Aisen, 1999). Serum transferrin and ovotransferrin transport iron from biological fluids to target cells by receptor-mediated endocytosis and release iron in the mildly acidic milieu of the late endosome (pH 5.5). Lactoferrin also binds iron but it is not involved in receptor-mediated endocytosis. Its release of iron is nearly impossible at pH 5.5 and is optimized near pH 3.5. Lactoferrin is present in numerous bodily secretions (milk, tears, mucus, saliva) and it is also released by white blood cells at the site of pathogen attack. It provides a bacteriostatic effect at these sites by scavenging free iron that could be used by bacteria, viruses, and fungi for growth (Abdallah and El Hage Chahine, 2000). A bacteriostatic function for MYP is also proposed by Cervello et al. (1994). They report that sea urchin coelomocytes release MYP under conditions of stress and perhaps this involves scavenging of iron ions from bacterial pathogens.

We have not yet examined the effects of pH on iron binding and release by MYP. In our in vitro iron binding assays we stripped iron from MYP at pH 5.5 (Brooks and Wessel, 2002). If MYP is endocytosed and packaged into yolk platelets with iron still bound, the acidification of the yolk platelets during development might serve as a timed release mechanism for this metal. The pH profile of sea urchin yolk platelets has been measured in vivo (Mallya et al., 1992) and during development the pH of this organelle shifts from pH 7.0 to 6.1. This drop in pH correlates to yolk protease activation and MYP processing and perhaps results in the release of iron. We can not exclude the possibility that iron doesn’t get packaged in the yolk platelet, but rather it is released from MYP early during endocytosis in an acidic endosome.

Nothing is known about the mechanism of uptake of MYP into oocytes. We assume that MYP is taken up by receptor-mediated endocytosis. Histological observations have revealed that much of the steady growth of the sea urchin ovary occurs by the accumulation and storage of MYP in the accessory cells. During vitellogenesis the accessory cells regress and transfer their contents to the oocyte, which endocytose the material and package it into membrane bound yolk platelets. The active transport of these molecules is corroborated by endocytic pits along the surface of vitellogenic oocytes that are observed under the electron microscope during vitellogenesis (Takashima and Takashima, 1966; Tsukahara and Sugiyama, 1969). The pH changes between endosomal compartments and the mature yolk platelet, if any, are not yet known.

YP30 is an oocyte-specific yolk protein in sea urchin

While the major yolk protein has attracted significant scrutiny, the other protein constituents of the sea urchin yolk platelet have received less attention and there is little data available. For this reason, another goal of our research is to identify and clone genes for additional protein constituents of the yolk platelet. We have recently reported the cloning and characterization of a unique oocyte-specific yolk protein that is synthesized exclusively within the oocyte (Wessel et al., 2000). To our knowledge, this is the first
Yolk protein dynamics

acetamine M. Brooks & Gary M. Wessel

Autosynthetic yolk precursor that has been cloned.

In most animals yolk protein precursors are derived from an extra-oocyte source, a process known as heterosynthesis, which provides the bulk of material for yolk formation. These animals include frog, chicken, nematode, fish, and some insects (Wallace, 1978; Kimble and Sharrock, 1983; Sharrock, 1984; Byrne, 1989). In other animals, mainly invertebrates, including molluscs, polychaetes and crustaceans, yolk is also made within the forming oocyte, a process known as autosynthesis (Ganion and Kessel, 1972; Bottke, 1973; Hill and Bowen, 1976; Lui and O’Conner, 1977; Bilinski, 1979; Eckelberinger, 1979; Zerbin, 1980). The ultrastructural study of Xenopus oocytes suggests that oocytes make a small contribution to yolk synthesis that begins before heterosynthesis (Kress, 1982). In all cases this combined production of yolk by extra- and intra-oocytic structures remains controversial as most of the studies where performed by ultrastructural analysis and have not been confirmed by molecular means.

Yolk formation in the sea urchin involves a combination of autosynthetic and heterosynthetic mechanisms. We have isolated a cDNA from two species of sea urchin that encodes yolk protein that we refer to as YP30 (30 kDa protein of the yolk platelet) that is synthesized exclusively by oocytes and packaged into yolk platelets (Wessel et al., 2000). This protein contains two repeated 150 amino acid domains with significant similarity to the repeats that were first found in fasciclin I, the neural cell adhesion protein identified from Drosophila and the grasshopper (Zinn et al., 1988). With regard to structure, fasciclin I has four fasciclin repeats while YP30 only possesses two. Interestingly, by yeast two hybrid analysis, we have shown that YP30 binds itself and forms dimers that give the complex four fasciclin-like repeats (Wessel et al., 2000). We hypothesize that YP30 may participate in the packaging and storing of MYP during oogenesis. We now have a full length MYP sequence and could test for a direct interaction with YP30 in the yeast system.

Utilization of the major yolk protein and YP30

Both MYP and YP30 accumulate to high levels during vitellogenesis. MYP has been estimated to be the most abundant protein in the sea urchin egg (Kari and Rottman, 1980), but by our estimation, YP30 is in molar excess in the yolk platelets of the unfertilized egg (Wessel et al., 2000). During early development, the profile of these two proteins is disparate. We estimate a 20-fold reduction of YP30 from egg to platelet and by comparison, MYP mass remains constant even though there is a change in composition as the protein goes through a series of step-wise proteolytic cleavages (Lee et al., 1989; Scott and Lennarz, 1989). We would like to understand the significance of degradation of YP30 during early embryogenesis. Perhaps YP30 is the vitelligenic resource of amino acids. It is a female-specific protein that is packaged in yolk granules and depleted during early embryogenesis. A point of consternation is also apparent with this observation, with depletion of YP30 so evident, how can the reported mass of the yolk platelet remain constant?

Yolk packaging

MYP is made in the gut and shuttled to the oocyte for packaging into yolk. Coincident with MYP synthesis and mobilization is the synthesis of another yolk
component, YP30, within the oocyte itself (Fig. 11). During the vitellogenic phase of oogenesis, MYP is endocytosed at the periphery of the oocyte and YP30 is targeted to

Figure 11. Model of the packaging of the major yolk protein and YP30 during vitellogenesis in sea urchin. (Adapted from Raikhel and Dhadialla, 1992).
During the vitellogenic phase of the oocyte and YP30 is targeted to

the secretory pathway of the oocyte. During this transit, MYP might release iron for its incorporation into metalloproteins forming within the oocyte, such as the ovoperoxidase. Since eventually only one population of yolk platelets exists in the egg, the pathways of yolk formation are not strictly separated. Since the yolk platelet protease has been shown to have proteolytic capacity within yolk platelets of the egg, it may be targeted during this same time frame. It could be made within the oocyte, like YP30, or it could be imported like MYP. The yolk platelets are stored in the egg within the ovary until the animal is stimulated to release them. The yolk remains intact during this storage period and is not utilized until the eggs are released, fertilized, and the embryo begins to develop. During early embryogenesis the yolk platelets become acidified and the protease(s) are activated. MYP is cleaved into smaller fragments and this may result in the release of iron that could then be incorporated into forming metalloenzymes. During this same time frame YP30 is degraded and could serve as a source of nutrition for the early embryo.

Concluding remarks

One of the major goals of our research is to identify and characterize yolk proteins in the sea urchin with the concomitant expectation that this information will increase our understanding of yolk platelet biology. The precise function(s) of these abundant organelles, present in the sea urchin embryo throughout early development, is unknown. Identifying the repertoire of proteins that comprise these organelles should help us elucidate their function.

In addition, biochemical studies are simplified due to the innate biology of this protein. MYP is highly enriched and easily isolated from both the coelomic fluid of adult sea urchins and the yolk platelets of eggs. The spatial dynamics of this coelomic fluid enrichment make in vivo experiments both plausible and attractive. We have been able to isolate MYP from the coelomic fluid, fluorescently tag it, and subsequently reintroduce it back to the sea urchin by a simple coelomic cavity injection. This has proved to be a useful technique allowing for in situ investigation of yolk protein biology including vitellogenic targeting and uptake of yolk protein precursors (Brooks and Wessel, manuscript in preparation).

Sea urchins have several experimental advantages for such studies. First, oocyte growth is asynchronous and oocytes at all stages of development are found adjacent to one another within the inner epithelium of the same ovary lobule. Second, oocytes are easily dissected and cultured, optically clear, and endocytically active. Third, near full grown oocytes are capable of spontaneous in vitro maturation and subsequent fertilization (Berg and Wessel, 1997). Finally, due to gamete size, all of these events can be directly visualized by either light (Fig. 12) or laser scanning confocal microscopes.

We hope that by addressing some of the questions outlined in the body of this review, we will gain a better understanding of the vitellogenic phase of oocyte growth and the dynamics of yolk platelet biology. The cloning and characterization of two distinct yolk proteins, MYP and YP30, should allow us to address yolk granule biogenesis including the extra- and intra-cellular contributions to yolk synthesis and the interactions involved in protein targeting and packaging to this population of discrete subcellular organelles.
Figure 12. Sea urchin oogenesis. These samples were dissected from the ovary of *Lytechinus variegatus* and viewed with a light microscope (panel A = 100X; panels B-I = 400X). (A) Oocytes attached to the somatic cells of the ovary. (B) Previtellogenic primary oogonia. (C-E) Vitellogenic growth phase of oogenesis. (E-I) Full grown oocytes complete meiosis with their large germinal vesicle moving asymmetrically to the cell periphery where it breaks down and extrudes two polar bodies to produce a haploid mature egg that is now ready for fertilization.

Abbreviations
HDLp, high-density lipoprotein; Lp, lipophorin; LpR, lipophorin receptor; LDLp, low-density lipoprotein; MYP, major yolk protein; NP, nutritive phagocyte; vHDLp, very-high-density lipoprotein; Vg, vitellogenin; VgR, vitellogenin receptor; YP, yolk protein; Y1, yolklcss

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Volk protein dynamics

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