Regulation of the Vitellogenin Receptor during Drosophila melanogaster Oogenesis

Christopher P. Schonbaum,* John J. Perrino, and Anthony P. Mahowald†

University of Chicago, Department of Molecular Genetics and Cell Biology, Chicago, Illinois 60637

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In many insects, development of the oocyte arrests temporarily just before vitellogenesis, the period when vitellogenins (yolk proteins) accumulate in the oocyte. Following hormonal and environmental cues, development of the oocyte resumes, and endocytosis of vitellogenins begins. An essential component of yolk uptake is the vitellogenin receptor. In this report, we describe the ovarian expression pattern and subcellular localization of the mRNA and protein encoded by the Drosophila melanogaster vitellogenin receptor gene yolkless (yl). yl RNA and protein are both expressed very early during the development of the oocyte, long before vitellogenesis begins. RNA in situ hybridization and lacZ reporter analyses show that yl RNA is synthesized by the germ line nurse cells and then transported to the oocyte. Yl protein is evenly distributed throughout the oocyte during the previtellogenic stages of oogenesis, demonstrating that the failure to take up yolk in these early stage oocyte is not due to the absence of the receptor. The transition to the vitellogenic stages is marked by the accumulation of yolk via clathrin-coated vesicles. After this transition, yolk protein receptor levels increase markedly at the cortex of the egg. Consistent with its role in yolk uptake, immunogold labeling of the receptor reveals Yl in endocytic structures at the cortex of wild-type vitellogenic oocytes. In addition, shortly after the inception of yolk uptake, we find multivesicular bodies where the yolk and receptor are distinctly partitioned. By the end of vitellogenesis, the receptor localizes predominantly to the cortex of the oocyte. However, during oogenesis in yl mutants that express full-length protein yet fail to incorporate yolk proteins, the receptor remains evenly distributed throughout the oocyte.

INTRODUCTION

The magnitude of yolk uptake into the oocyte during vitellogenesis suggests a heavy involvement of the endocytic machinery; indeed, the clathrin-coated vesicle was originally described in the vitellogenic mosquito oocyte (Roth and Porter, 1964). Because the morphological features are so striking, descriptions of vitellogenesis have been made in a broad range of oviparous species, including birds (Perry and Gilbert, 1979; Perry et al., 1984), frogs (Opresko and Wiley, 1987; Wall and Patel, 1987), fish (reviewed by Wallace and Selman, 1990), and insects (Cummings and King, 1970; Mahowald, 1972; Giorgi and Jacob, 1977a,b; Raikhel, 1984; van Antwerpen et al., 1993) (reviewed by Raikhel and Dhadialla, 1992). In several cases, immunocytochemical and ultrastructural studies using labeled yolk protein precursors or fluid phase markers have followed the fate of the proteins as they are sorted to the yolk platelets (Giorgi and Jacob, 1977a; Raikhel, 1984; Busson et al., 1989). 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 (Vgs) are taken up through clathrin-coated pits, and they accumulate initially in vesiculotubular structures (early endosomal structures), which coalesce into primary yolk bodies (analogous to late endosomal structures). In contrast to general endocytic pathways where the internalized ligands are degraded in lysosomes, yolk proteins are stored as yolk granules for later use during embryogenesis. The yolk granules appear to be modified lysosomes with relatively high pH; during embryogenesis, the pH of the yolk granule drops to levels more typical of lysosomes (Fagotto, 1995).

Until recently, the location of the vitellogenin receptor (VgR) throughout this process had not been examined directly. Generally, the fate of the receptor had been inferred by following fluid phase markers and labeled vitellogenins. Tubules labeled with the fluid phase marker but not the yolk proteins were suggested to be receptor recycling tubules by analogy to the morphologically similar structures identified as recycling tubules in other endocytic systems (Geuze et al., 1997).
VgRs now permits such an analysis. Shen et al. (1993) found the chicken VgR in endocytic structures (coated pits, vesicles, and tubules); however, recycling compartments were not described. Snigirevskaya et al. (1997) also described endocytic compartments and putative recycling compartments that contained the mosquito VgR. In this study, we undertook an analysis of the Drosophila yolk protein receptor distribution during oogenesis. We address the relationship of the expression and intracellular distribution of the receptor to the development of the oocyte and to the onset of vitellogenesis. Moreover, the availability of yl mutants in Drosophila has enabled us to examine the distribution of receptors defective in yolk uptake.

VgRs from birds, insects, fish, and frogs (reviewed by Schneider, 1996; Sappington and Raikhel, 1998), all belong to the low-density lipoprotein receptor superfamily. The conservation of vitellogenin receptors across such diverse phyla suggests a conserved mechanism not only in yolk uptake but also potentially in the regulation of vitellogenin receptors. For example, in many insects, oocyte development arrests just before Vg uptake. Hormonal and environmental cues induce the oocyte to resume development and to begin vitellogenesis. Juvenile hormone (JH), in particular, seems to play a key role in various insects in releasing the oocyte from this block (reviewed by Raikhel and Dhadialla, 1992). Recent work also suggests that ecdysteroids may be involved in the inception of vitellogenesis (Richard et al., 1998); however, the cellular mechanisms underlying the initiation of yolk uptake are unresolved. Thus, in addition to the endocytic profile, we were interested in the regulation of yl during oogenesis. In this paper, we describe the expression patterns of yl RNA and protein in normal flies as well as in mutants defective in oogenesis. Based on this analysis, we address the relationship of the expression and intracellular distribution of the yl RNA and protein to the development of the oocyte and the onset of vitellogenesis. We also identify gene regulatory regions sufficient for germ line expression of yl.

MATERIALS AND METHODS

Fly Culture

Drosophila cultures were maintained at 24°C on standard cornmeal molasses agar unless otherwise specified. All yl alleles have been described previously (DiMario et al., 1987). We thank Rod Nagoshi (University of Iowa) for sending the female sterile yl alleles generated by J. Mohler (1977), Beat Suter (McGill University) for the BicD flies, and Tom Wilson (Colorado State University) for the ap4 flies. We also thank Julie Feder for assistance in use of the confocal microscope.

RNA In Situ Hybridization

Whole-mount RNA in situ hybridization was carried out as described by Tautz and Pfeifle (1989) with modifications of the methods of Ephrussi et al. (1991). Random-primed digoxigenin-labeled DNA probes were prepared as described by the manufacturer (Boehringer Mannheim, Indianapolis, IN). The yl cDNAs used for the in situ hybridizations have been described previously (Schonbaum et al., 1995).

yl-lacZ Reporters

To construct the CHZY191 (−20/−400) line, a 375-bp region, from −20 to −395 bp upstream of the strong yl transcription start site (our unpublished results), was generated by PCR amplification and cloned into the pCasper-hs43-lacZ Vector (Thummel and Pirrotta, 1991). The sequence of the 230-bp amplified region was confirmed. The CHZY195 (−20/−1700) line was made by adding a 1.3-kb BamHI fragment (−395/−1685) to the CHZY191 construct and isolating the clone with the fragment inserted in the correct orientation. The CHZY196 line was prepared by cloning a SphI-BamHI (−215/−1685) into the casper-hs43-lacZ vector. DNAs were purified (Midipreps; Qiagen, Hilden, Germany) and coprecipitated with a helper P transposase plasmid (pHsp). DNAs were injected into yw111B hosts and selected for white+ phenotype as described previously (Schonbaum et al., 1995). Transgenic lines were tested for β-galactosidase activity with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (Margolis and Spradling, 1995).

Immunostaining

Whole-mount protein immunostaining was modified from a procedure provided by H. Rusohola-Baker (Rusohola et al., 1991). Ovaries were dissected in PBS and fixed for 15 min to 1 h with 4% paraformaldehyde in PBS. The ovaries were washed four times with PBT (PBS plus 0.1% Tween 20) and once with PBTXT (PBT plus 0.3% Triton X-100, 0.1% Tween 20) and blocked for 1–2 h at room temperature with PBTXT plus 2% BSA (Sigma, St. Louis, MO). The ovaries were incubated with anti-Yl whole serum (1:100–1:300, diluted in PBTXT + 2%BSA) or affinity-purified anti-Yl antibodies (1:100) overnight at 4°C. The rat anti-Yl antibodies have been described previously (Schonbaum et al., 1995). The ovaries were washed extensively with four 1.5-h washes with PBTXT and then incubated overnight at 4°C with preabsorbed secondary antibody (fluorescein-conjugated goat anti-rat immunoglobulin, diluted 1:400–1:500 in PBTXT). The secondary antibodies had been preabsorbed with affinity-purified rat anti-Yl antibody diluted 1:150 in blocking solution. Sections were washed three times for 10 min each in PBS, incubated in a humidified chamber at room temperature for 2% BSA or affinity-purified anti-Yl antibodies overnight at 4°C with preabsorbed secondary antibody (fluorescein-conjugated goat anti-rat immunoglobulin, diluted 1:400–1:500 in PBTXT). The secondary antibodies had been preabsorbed for >2 h against fixed embryos. Finally, the ovaries were washed again four times with PBTXT, rinsed with PBT, and mounted in Aquamount (Polysciences, Warrington, PA) or in 70% glycerol. Samples were viewed on a Zeiss (Thornwood, NY) Laser Scan confocal microscope.

Immunogold Labeling

Ovaries were dissected and fixed in 0.1 M NaPO₄, pH 7.4, containing 4% formaldehyde (electron microscopy [EM] grade; Electron Microscopy Sciences, Fort Washington, PA) for 10 min at room temperature and then 1 h at 4°C. For thin sections two techniques were used. For cryosections, fixed samples were washed three times in 0.2 M sucrose in 0.1 M NaPO₄, pH 7.4, and then allowed to equilibrate in 20% polyvinylpyrrolidone in 1.84 M sucrose. Thin sections were collected on tungsten loops with 2.3 M sucrose and mounted on Formvar- and carbon-coated nickel grids. For plastic sections, fixed samples were washed three times for 5 min with 0.1 M NaPO₄, dehydrated through a graded ethanol series at decreasing temperatures, infiltrated with graded alcohol and Lowicryl K4M resin according to the manufacturer’s instructions (Electron Microscopy Sciences) at −25°C. Samples were polymerized using UV light for 3–5 d at −25°C. Ultrathin sections were mounted onto Formvar- and carbon-coated nickel grids. For immunostaining all solutions were centrifuged briefly (3000 rpm for 5 min) or were clarified with 0.2-μm filters. Sections were washed in PBS for 15 min and blocked for 3 h at room temperature with 2% BSA in PBS. They were then incubated in a humidified chamber at room temperature for 2 h with affinity-purified rat anti-Yl antibody diluted 1:150 in blocking solution. Sections were washed three times for 10 min each in PBS drops and then incubated for 2 h with 15-nm gold-conjugated goat anti-rat immunoglobulin G (Amersham, Arlington Heights, IL) diluted 1:5 in blocking solution. After washing in PBS as before, the sections were postfixed briefly with 1% glutaraldehyde in PBS and then washed briefly with water. Finally, the plastic sections were stained with uranyl acetate and lead citrate, and the cryothin sections were stained and dried in 2% polyvinylalcohol with 0.03% uranyl acetate.
complex has been enveloped by follicle cells (Figure 2A, inset). The RNA is concentrated in a single cell within the 16-cell cyst. This cell is clearly the oocyte in region 3 (stage 1) ovarian cysts, and we assume that the single cell accumulating yl RNA in regions 2A and 2B is the presumptive oocyte.

During later stages of wild-type oocyte development, yl RNA continues to be found in the oocyte (Figure 2B). However, in contrast with the earlier stages of development, yl RNA levels become more pronounced in the nurse cells of stage 9 and 10 chambers. There does not appear to be any specific localization of the RNA within the oocyte at any time.

Transport of nurse cell-derived RNAs into the oocyte has been noted for several genes important in the development of the oocyte (e.g., BicD [Suter et al., 1989], osk [Ephrussi et al., 1991; Kim-Ha et al., 1991]; fs(1)K10 [Cheung et al., 1992], CycB [Dalby and Glover, 1992], and orb [Lantz et al., 1992]). Transport of yl RNA into the oocyte is also suggested by examination of yl RNA distribution in egl and BicD mutants. In both of these mutants, the oocyte does not differentiate normally, and instead of 15 nurse cells and an oocyte, a cluster of 16 nurse cells is formed. yl RNA is detected in the germ line of early stage egl and BicD chambers (Figure 2, C and D); however, the RNA is now distributed almost equally among the 16 cells. Some yl RNA localization is still evident in the hypomorphic BicD<sup>D<sub>Age</sub></sup> (Figure 2D) and the BicD<sup>R<sub>26</sub></sup> alleles (our unpublished results), even though the posteriorly positioned cell does not fully develop into an oocyte. Similar effects on osk and orb RNA localization have been observed in BicD and egl mutants (Ephrussi et al., 1991; Ran et al., 1994).

yl-lacZ reporter gene constructs confirmed that yl RNA is transcribed in the nurse cells. Furthermore, they identified a minimal enhancer region sufficient for germ line-specific expression of yl. Previously, a genomic DNA fragment that contained the yl gene, including a region 1.7 kb upstream of the start site, was shown to be sufficient for rescue of the yl mutant phenotype (Schonbaum et al., 1995). We cloned portions of this 1.7-kb region into a reporter vector bearing an hsp70 basal promoter element linked to the lacZ gene (Thummel and Pirrotta, 1991). The β-galactosidase expressed from these constructs accumulates in the nucleus because of the presence of a nuclear localization signal. Transgenic animals bearing either the 1.7-kb region (our unpublished results) or the first 400 bp upstream of the yl start site (Figure 2F) fused to the reporter showed β-galactosidase activity in all nurse cell nuclei. Animals with a 200-bp region upstream of the transcription start site also exhibited germ line-specific expression of the reporter; however, the signal was weaker and somewhat variable (our unpublished results). Transgenic lines with 1.5 kb of upstream sequences but lacking the first 200 bp upstream of the transcription start site did not show any ovarian β-galactosidase staining (our unpublished results).

Yolkless Protein Expression

The Yl protein distribution mirrors the RNA pattern in previtellogenic stages of oocyte development (Figure 3A). Yl can be detected by stage 1 (region 3 in the germarium; cf. Figure 1). Like the RNA pattern, the protein is concentrated in the oocyte. Optical sectioning of wild-type chambers by
confocal microscopy indicates that Yl protein is diffusely distributed throughout the oocyte up through stage 7 (Figure 3B). Although the majority of the Yl protein is in the oocyte, we can detect some Yl in nurse cells adjacent to the oocyte (Figure 3A, inset; our unpublished results). Before the vitellogenic stages, endocytic structures, such as coated vesicles, are not found along the oocyte–follicle cell border (Figure 4; Mahowald, 1972). However, soon after the transition to the vitellogenic stages, defined as stage 8 (Cummings and King, 1969), endocytic structures become prominent (Mahowald, 1972). Yl can be seen accumulating at the surface (cortex) of the stage 8–9 oocyte (Figure 3C) with moderate Yl staining in the internal regions of the oocyte. As oogenesis proceeds, the cortical staining intensifies, and optical sectioning by confocal microscopy shows little Yl present within the center of the stage 10 oocytes. By the end of stage 10, the receptor is almost exclusively cortical (Figure 3D). In addition, at stage 10, there appears to be little Yl protein in the nurse cells, even though Yl RNA levels in the nurse cells are elevated (Figure 2B).

**Figure 2.** Yl RNA expression in the germ line cells. (A) Whole-mount RNA in situ hybridization shows Yl RNA is expressed in wild-type germ line cells and accumulates in the oocyte as early as region 2 in the gerarium (g). Inset, Higher magnification of a gerarium with Yl RNA localized to the presumptive oocyte of region 2 and region 3 cystocytes. (B) Yl RNA levels in wild-type ovaries increase in the nurse cells during stages 9 and 10. (C) In egl1/egl1 mutant chambers, which have 16 nurse cells but no oocyte, Yl RNA is still expressed but it is not localized to any of the cells. (D) Similar results are seen with a hypomorphic BicD allele [BicD<sup>1ac</sup>/Df(2)TW119], although some RNA accumulation in the most posterior cell is seen in very early stage chambers. (E) Staining is not seen in the Yl null [Df(1)Yll/Df(1)KA2] control. (F) β-Galactosidase is detected in the germ line cells of a Yl-lacZ transgenic ovary. The Yl-lacZ reporter construct contains the first 400 bp upstream of the Yl transcription start site.
ovaries (Figure 6D). Vitellogenesis is also blocked in certain
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expression. Hypomorphic mutants of the stand still (stil)
gene arrest oogenesis at a stage that resembles stages 9–10.

null controls. Bar, 10 μm.

Figure 3. Whole-mount in situ immunolocalization of Yl in wild-
type oocytes. (A) Whole-mount in situ immunolocalization shows
that the Yl protein is expressed in the previtellogenic stages. Expression
is uniform in the stage 1–5 oocytes. Inset, Protein levels in the
stage 2 chamber are highest in the oocyte, but lower levels are
apparent in the nurse cells just adjacent and connected to the oocyte
via ring canals. (B) In a stage 7 chamber, before vitellogenesis has
begun, the receptor is distributed throughout the oocyte. (C) A stage
9 chamber, where vitellogenin is being accumulated, shows Yl
enrichment at the cortex of the oocyte. (D) By the end of stage 10, the
protein appears to be mostly cortically localized. (E) Df(1)KA9 yl
null controls. Bar, 10 μm.

Cortical accumulation of the Yl protein can be disrupted
by mutations in yl. Several alleles (yl15, yl19, yl20, and yl21),
have been identified in which full-length 210-kDa protein is
synthesized (Figure 5), but the protein remains distributed
throughout the oocyte (Figure 6, A–C). These mutants fail to
accumulate appreciable levels of yolk proteins and they
have dramatically reduced numbers of endocytic structures
(DiMario et al., 1987). Females bearing weak yl alleles (yl?),
which have reduced but still significant levels of yolk pro-
tein accumulation (DiMario et al., 1987), exhibit cortical lo-
calization of the receptor (our unpublished results).

Mutations in other genes with effects on vitellogenesis
have been described. We tested two of these for effects on Yl
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ovaries (Figure 6D). Vitellogenesis is also blocked in certain
apterous (ap) mutants with depressed JH levels. apterous acts
nonautonomously to affect ovary development. Oocytes in
ap mutants arrest at stage 7, and yolk uptake is not seen
(Postlethwait, and Weiser, 1973). Administration of juvenile
hormone restores oocyte development and yolk uptake
(Postlethwait, and Weiser, 1973; Gavin and Williamson,
1976). In arrested 1- to 2-d ap oocytes, Yl expression is
uniformly distributed, as in wild-type previtellogenic oo-
cyes (our unpublished results).

Subcellular Distribution of Yl during Vitellogenesis
We next studied the distribution of Yl at the ultrastructural
to clarify the relocalization of Yl during the transition
from pre- to postvitellogenic stages of oogenesis. Using im-
munogold staining of sections of ovarian chambers embed-
ded in Lowicryl or cryosections, we have been unable to
detect the Yl receptor in egg chambers before the inception
of yolk uptake. This is surprising, especially because the
receptor was readily detected at the light microscopic level
in these stages after similar paraformaldehyde fixation. In
contrast, our EM immunogold staining methods were able
to detect Yl in vitellogenic oocyte stages (Figures 7 and 8A).
Colloidal gold particles were detected in both endocytic and
tubular structures in the cortex of the oocyte. More inter-
nally, Yl is detected around the perimeter of immature yolk
spheres and in tubular-like projections adjacent to the yolk
granules. The receptor is also found in the flocculent asso-
ciated body that lies to one side of the mature yolk sphere
(Figure 8B).

At the transition to stage 8, when endocytosis of yolk is
beginning, multivesicular bodies (MVBs) are heavily labeled
for Yl (Figure 9). In some instances, the receptor is inter-
erspersed with an electron-dense material (Figure 9A); in other
cases, the electron-dense mass has coalesced, and the recep-
tor is segregated to the periphery of the MVB (Figure 9B).
The dense mass found in these MVBs reacts positively for
yolk proteins (our unpublished results). These multivesicu-
lar structures are abundant in early vitellogenic stage cham-
bers but are found more rarely in stage 9–10 oocytes. They
always show a strong staining with the Yl antibody. Yl
staining of the oocyte cortex is found in both early and late
vitellogenic stages.

Cortical staining is abolished in yl mutants that express
relatively high levels of a full-length mutant Yl protein.
Instead, the defective protein was uniformly distributed and
appeared to reside predominantly in the endoplasmic retic-
ulum (Figure 10). Similar results were seen for the yl20 and
yl19 mutants.

DISCUSSION

Yl Expression
Based on the yolkless mutant phenotype (DiMario et al., 1987)
and on similarity of the sequence of the yolkless gene to the
vertebrate vitellogenin receptor, we proposed previously
that yolkless encoded a vitellogenin receptor in Drosophila
mellonaster (Schonbaum et al., 1995). This was subsequently
confirmed by the cloning of a very closely related vitellege-
nin receptor from another dipteran, the mosquito Aedes ae-
gypti (Sappington et al., 1996). The similarity of vitellogenin
receptors from insects to birds suggests conserved mecha-

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nisms for regulating yolk uptake into eggs. We have now analyzed the expression patterns of *yl* RNA and protein to identify potential regulatory steps during *Drosophila* vitellogenesis.

RNA in situ hybridization, Yl immunolocalization, and *lacZ* reporter studies showed that *yl* RNA and protein are expressed in germ line cells very early during oogenesis, long before the protein is required for vitellogenin uptake. Clearly, expression of the vitellogenin receptor is not the limiting component for yolk uptake. The *lacZ* reporter studies also identified sequences in the first 400 bases upstream of the transcription start site that are necessary and sufficient to direct expression in the germ line cells. We have not ruled out additional regulatory elements downstream of the transcription start site. It will be interesting to see whether vitellogenin receptor genes from other species are regulated by a conserved set of germ line transcription factors.

The expression studies also showed that *yl* RNA is transcribed in the nurse cells and then transported into the oocyte, as has been seen with a number of other *Drosophila* genes that have roles in oogenesis (reviewed by Lasko, 1999). Transport of *yolkless* RNA into the oocyte likely occurs via a microtubule-based transport system that has been implicated in the movement of other RNAs from the nurse cells into the oocyte (Cooley and Theurkauf, 1994). RNAs pro-

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**Figure 4.** Wild-type previtellogenic oocyte. (A) Electron micrograph of a stage 6 oocyte, showing the characteristic spherical oocyte nucleus (N), the absence of coated vesicles along the follicle cell (F)–oocyte (O) border (shown at higher magnification in B), and the presence of a dispersed endoplasmic reticulum (arrows), which is absent from nurse cells (NC). Magnification: A, 8100×; B, 42,000×.

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**Figure 5.** Western blot analysis of *yl* mutants. Western analysis of ovary proteins using anti-Yl antibodies shows that the 210-kDa Yl protein is absent in *yl* alleles with a strong phenotype (*yl*11, *yl*14, *yl*28), but it is present in other alleles with strong reductions in yolk uptake (*yl*15, *yl*29, *yl*21) as well as in alleles with weak phenotypes (*yl*18). Two alleles, *yl*16 and *yl*29, expressed truncated proteins of 130 kDa (*) and 175 kDa, respectively. The low-molecular-weight band is a cross-reacting protein. It is not detected using affinity-purified anti-Yl antibodies; however, it serves as a useful loading control.

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**Figure 6.** Yl distribution in yolk uptake mutants. In strong *yl* mutants, which express full-length Yl protein yet fail to take up yolk, the Yl protein does not become cortically localized during the vitellogenic stages. (A) Early stage 9 *yl*21 oocyte; (B) stage 10 *yl*21 oocyte; (C) stage 10 *yl*15 oocyte. (D) Cortical Yl localization also fails to occur in *stil*115*Ve*2 mutant ovaries. Bar, 20 μm.
duced by the oocyte nucleus do not depend upon the microtubule transport system (Saunders and Cohen, 1999). Recent surveys of *Drosophila* genes indicate that up to 10% of germ line-expressed RNAs may be transported into the oocyte, but patterns of transport vary between genes (Dubowy and Macdonald, 1998). Some RNAs are transported efficiently and early during oocyte differentiation, whereas others are transported slowly or later and do not accumulate in the oocyte until late previtellogenic stages or postvitellogenic stages. *yolkless* falls into the class of genes whose RNA is transported efficiently and very early. We see no obvious localization of the *yl* RNA to a particular region of the oocyte; thus, the *yl* RNA should possess sequences solely involved in RNA transport. Distinct RNA transport and localization signals have been identified in the 3′ untranslated RNA sequences of nanos and *osk* RNAs (Kim-Ha et al., 1993; Gavis et al., 1996). It will be interesting to compare the regions of *yl* RNA that are required for transport to those of other oocyte localized RNAs.

**Yl Localization**

Consistent with its proposed role as a vitellogenin receptor, Yl protein is present at the cortex of vitellogenic stage oocytes. Yl was seen in coated vesicular and tubular (early endosomal) structures. Yl was also seen associated with smaller yolk granules where the receptor was present in tubular projections. These projections likely represent a sorting and recycling tubule. In more mature yolk granules, Yl labeling at the perimeter of the granule may represent the fraction of the receptor that was not recycled. The mature yolk granule appears to be a modified lysosome with reduced hydrolytic activity (Fagotto, 1995). Thus, receptors that were not recycled would end up associated with the yolk granule.

In contrast to the vitellogenic stages, Yl is uniformly distributed through the oocyte during previtellogenic stages. During these stages, there is no evidence of endocytosis at the oocyte–follicle cell border, and oocytes do not internalize vitellogenins. The failure to take up yolk in previtellogenic stages does not result from the absence of the receptor protein. Is yolk uptake caused by relocation of the receptor? Redistribution of vitellogenin receptors upon the onset of vitellogenin uptake is also seen in chickens. In small previtellogenic chicken oocytes, the VgR is initially detected in vesicular structures within the interior of the oocyte, with little receptor present at the cell surface (Shen et al., 1993). However, during the phase of rapid vitellogenin uptake, the

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**Figure 7.** Ultrastructural localization of Yl at the oocyte cortex. Immunoelectron micrograph of cryothin sections of the cortex of a stage 10 oocyte, stained with 5-nm colloidal gold-labeled secondary antibodies to Yolkless antibody. Antigen is present in coated vesicles (v) and tubules (t), as well as at the surface of nascent yolk platelets (arrow). The vitelline membrane (VM) is at the top. Magnification, 84,000×.
chicken receptor relocalizes mainly to the cortex of the oocyte. When the subcellular distribution of Yolkless was examined to determine whether the receptor was present in a specific compartment during previtellogenic stages, we were unable to detect Yl using immunogold techniques, even though by confocal microscopy, we could see that Yl was distributed throughout the oocyte. Although there appears to be lower protein levels in previtellogenic stages, the whole-mount results still suggest that the protein was abundant enough to be observed by EM immunolabeling, especially by stage 7. In addition, immunogold labeling of mutant Yl receptor in internal regions of vitellogenic oocytes (Figure 10) suggests that the inability to detect Yl in previtellogenic stages reflects a difference between Yl synthesized during previtellogenic stages and that synthesized during vitellogenic stages. We propose that Yl protein is masked before the transition to vitellogenesis, possibly in the relatively abundant endoplasmic reticulum in the oocyte (Figure 4).

The mechanism underlying VgR relocalization in insects and birds is unknown. One example of regulated endocytosis is seen in the response of mammalian adipose and muscle cells to insulin. Insulin stimulates relocalization of the GLUT4 glucose transporter to the cell surface. Before reception of the signal, the GLUT4 receptor is enriched in intracellular vesicles (reviewed by Pessin et al., 1999). The redistribution of GLUT4 appears to be a case of regulated exocytosis, as occurs during synaptic vesicle fusion; it may also involve the selective retention of the transporter within a distinct intracellular compartment. Interestingly, the GLUT4 C-terminal domain appears to be masked in Lowicryl sections before the insulin-stimulated redistribution (Wang et al., 1996). Is a similar mechanism regulating vitellogenin uptake? Hormonal and/or environmental stimuli initiate yolk uptake in many insects (reviewed by Raikhel and Dhadiaalla, 1992). In particular, JH can stimulate vitellogenin uptake both in vivo and in vitro in many insects, although its mechanism of action in the ovary is unknown (Tedesco et al., 1981; Raikhel and Lea, 1985). Consistent with this role for JH, Yl is not cortically localized in JH deficient...
apterous14 oocytes. It is not known whether yolk uptake in vertebrate systems is hormonally mediated.

Unlike the GLUT4 system, the onset of vitellogenin uptake appears to involve regulation of general endocytosis, not just a specific receptor. Uptake of fluid phase markers such as ferritin, trypan blue, and horseradish peroxidase is not detected in previtellogenic ovaries of dipterans (Mahowald, 1972; Giorgi, 1979; Raikhel and Lea, 1985), suggesting a complete or significant reduction in endocytosis. Because the oocyte and the polar follicle cells signal via cell surface receptors during previtellogenic stages (Gonzalez-Reyes et al., 1997, Newmark et al., 1997; Gonzalez-Reyes and St. Johnston, 1998), there must be some membrane trafficking within the oocyte. Thus, there may not be a complete block in endocytosis. It is not clear, however, whether these signaling events are dependent on endocytic activity.

Although we were unable to detect Yl by immunogold labeling of previtellogenic oocytes, we were able to observe the protein easily in early vitellogenic stage chambers. In particular, multivesicular bodies were prominently labeled during the early stages. The presence of vitellogenin receptors in MVBs has not been reported in other ultrastructural studies that have examined receptor location during vitellogenin uptake (Shen et al., 1993, Snigirevskaia, 1997). However, MVBs have been noted in ultrastructural studies of insect vitellogenesis (Mahowald, 1972; Giorgi and Jacob, 1977a; Raikhel and Lea, 1986), and during Xenopus vitellogenesis, gold-labeled vitellogenin is incorporated into multivesicular bodies (Wall and Patel, 1987). MVBs are a distinct feature of general endocytosis (Mukherjee et al., 1997), where they are considered a late endosomal compartment.

We do not yet know the series of events by which Yl becomes associated with the MVB. By analogy to mamma-
Drosophila melanogaster


