Development 117, 319-328 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

Ultrastructural analysis of *Drosophila* ovarian follicles differing in yolk polypeptide (yps) composition

F. Giorgi[1,*], P. Lucchesi[1], A. Morelli[1] and M. Bownes[2]

[1]Department of Biomedicine, University of Pisa, Via A. Volta 4, 56100 - Pisa, Italy
[2]Department of Molecular Biology, University of Edinburgh, Scotland

*Author for correspondence

SUMMARY

***Drosophila* ovarian follicles were examined ultrastructurally to study the vesicular traffic in the cortical ooplasm. The endocytic pathway leading to the production of yolk spheres was visualized following in vivo or in vitro exposure to peroxidase. The Golgi apparatus and the yolk spheres of wild-type ovarian follicles were preferentially labelled by fixation with osmium zinc iodide (OZI). Labelling of wild-type ovarian follicles was compared to that of several mutant follicles - *L186/Basc, fs(2)A17* and *ap[4]* - which are defective in vitellogenesis. In these mutants, the Golgi apparatus and the vesicles nearby were either scantly labelled or not labelled at all. In oocytes from flies homozygous for the gene *fs(1)1163*, the Golgi apparatus was labelled as in the controls, but no yolk spheres appeared to be labelled with OZI at any of the developmental stages.**

In several *Drosophila* strains, the pattern of OZI label in the cortical ooplasm was seen to vary in relation to the number of yp structural genes. In starved *Drosophila* females, OZI labelling of the cortical ooplasm appeared restricted to the Golgi apparatus and to an extended tubular network. A similar labelling pattern was also detected in in vitro cultured vitellogenic follicles. Refeeding, topical application of juvenile hormone analogue to starved females or hormone addition to the culture medium, all caused the yolk spheres to become labelled with OZI and to incorporate peroxidase. These observations prove that impairing endocytic uptake by either mutation or lack of juvenile hormone prevents fusion of coated vesicles and tubules with the yolk spheres and leads them instead to form an intermediate cell compartment with Golgi-derived vesicles.

Key words: *Drosophila*, oogenesis, yolk polypeptide

INTRODUCTION

Vitellogenesis in insects occurs by receptor-mediated endocytosis of a yolk protein (YP) precursor or vitellogenin from the maternal haemolymph (Koenig et al., 1988; Ferenz, 1990; Dhadialla and Raikhel, 1991). YPs are internalized via coated pits and vesicles along the oocyte plasma membrane and transferred to early endosomes in the cortical ooplasm (Postlethwait and Giorgi, 1985; Raikhel and Lea, 1986). From this cell organelle, YPs are conveyed to a modified form of late endosomes, called yolk spheres (Mahowald and Kambysellis, 1980), while receptors are recycled back to the oocyte surface (Raikhel and Dhadialla, 1992). During transfer to the yolk spheres, YPs are processed to an insoluble, crystallized, storage protein or vitellin (Cummings and King, 1970; Giorgi and Jacob, 1977a,b).

Recently, Yan and Postlethwait (1990) have demonstrated that *Drosophila* YPs carry sufficient structural information to direct them to the yolk spheres. However, given the actual complexity of the vesicular traffic between the oocyte plasma membrane and the yolk spheres (Roth and Porter, 1964; Giorgi, 1980; Raikhel, 1984), other factors, besides the nature of the internalized ligand, may be expected to contribute to yolk sorting in vitellogenic follicles. It is known, for instance, that early endosomes in somatic cells will gradually transform into late endosomes upon interaction with lysosomes (Stoorvogel et al., 1991). Whether lysosomes are also present in vitellogenic oocytes is still a controversial issue. *Xenopus* oocytes have recently been shown to contain a type of lysosome with unusual enzyme activities (Wall and Meleka, 1985). Lysosomes in vitellogenic oocytes might perhaps help to process YPs into a storage form of vitellin, although exactly how they interact with yolk spheres is not yet clear.

The present work aims to clarify how the endocytic pathway that leads to the formation of yolk spheres involves lysosomal interaction during vitellogenesis. To achieve this, the endocytic pathway of *Drosophila* vitellogenic follicles was studied ultrastructurally following in vivo and in vitro exposure to peroxidase. Since lysosomes are known to emerge from the *trans* Golgi network (Griffiths and Simons, 1986), this study focuses on the structural relationship between the Golgi apparatus and the yolk spheres, as visu-

alized by fixation with osmium zinc iodide (Maillet, 1963; Niebauer et al., 1969). This analysis was also extended to examine vitellogenic oocytes that had been altered either numerically or by mutation in yp genes.

Vitellogenesis in insects is a hormone-dependent process (Belote et al., 1985; Tamura et al., 1985; Bownes, 1989). A secondary objective of the present study was thus to verify the oocyte's capability to undergo endocytosis and to convey newly endocytosed YPs to the yolk spheres in response to in vivo or in vitro administration of juvenile hormone (Bownes and Blair, 1986; Bownes et al., 1988). The results showed that the vesicular traffic in the cortical ooplasm is altered by conditions that affect the number and the structure of yp genes as well as the availabity of juvenile hormone to the ovary by either starvation or in vitro culture.

MATERIAL AND METHODS

Wild-type and mutant *Drosophila* flies were reared in glass bottles containing standard molasses-corn-yeast food and maintained at a constant temperature of 25°C. The following female sterile mutations were used: *L186/Basc, fs(2)A17, apterous*^{4} *and fs(1)1163*. Strains differing in yp gene number were produced through crosses between flies deleted or mutated in one of the three yp genes. The strains used in this study and the gene dosage per diploid set are indicated in Table 1. A number of wild-type 2- to 3-day-old female flies were allowed to starve for 24 hours in humidified bottles. At the end of this period, some were killed for ultrastructural and cytochemical analyses, while others were allowed to feed for additional 12 hours. The remaining starved flies were treated by topical application of the juvenile hormone analogue ZR-515 (Zoecon) in acetone.

Experimentally treated or mutant flies were injected with 0.2 μl of horseradish peroxidase (5 mg/ml Sigma) in sterile *Drosophila* Ringers solution. 2 hours after injection, flies were anaesthesized and the ovaries dissected in fixative. Freshly dissected vitellogenic ovaries were also cultured for 2 hours in Grace's medium in the presence of horseradish peroxidase (5 mg/ml) with or without ZR-515 (Giorgi, 1979). Fixation was prolonged for 2 hours in 5% glutaraldehyde-4% formaldehyde in 0.1 M cacodylate buffer at pH 7.2 at 4°C. Peroxidase activity was revealed by 30 minute incubation in diamino-benzidine (DAB) in 0.1 M Tris-HCl pH 6.8 containing 0.1% H_2O_2. Post-fixation was carried out for 2 hours in 1% osmium tetroxide in 0.1 M cacodylate buffer at pH 7.2. Ovaries were eventually dehydrated in a graded series of alcohols and embedded in Epon-Araldite. Polymerization was achieved by 3 days incubation at 60°C.

In a parallel series of experiments, 2- to 3-day-old ovaries from either wild-type, mutant or experimentally treated flies, were fixed in osmium zinc iodide (OZI) for 8 hours at room temperature according to Maillet (1963) as modified by Niebauer et al. (1969). Thin sections were prepared using an LKB ultramicrotome, stained conventionally in uranyl acetate and lead citrate, and examined in a 100SX Jeol electron microscope.

The extent of OZI labelling was evaluated quantitatively using a Quantimet 570 image analyzer (Cambridge Instruments). Various OZI-labelled organelles were differentially colour-contrasted and expressed as a percentage of the ooplasm area visualized by the image analyzer. In total, 27 μm^2 of OZI-labelled area, distributed over 21 pictures of equal photographic enlargement, were examined by this procedure.

Table 1. *Drosophila* strains and mutants with different gene dosages

STRAIN	YP-GENES	GENE DOSE
Oregon R	1 1 2 2 3 3	6
yp 2$^-$	1 1 - - 3 3	4
Df(1)C52/FM6	1 - 2 - 3 3	4
yp 3$^-$	1 1 2 2 - -	4
fs(1)1163	- - 2 2 3 3	4
Df(1)C52yp3$^-$/FM6	1 - 2 - 3 -	3
yp 2$^-$ yp 3$^-$	1 1 - - - -	2

Df(1)C52 carries a deletion for YP1 and YP2. *fs(1)1163* is a secretion mutant that lacks YP1 in haemolymph and eggs. *Yp2*$^-$ and *YP3*$^-$ are mutants that lack YP2 and YP3 respectively in haemolymph and eggs. The *FM6* balancer chromosome carries *Y sc dm* and *B*. The *fs(1)1163, YP2*$^-$, *YP2*$^-$*YP3*$^-$ and *Df(1)C52 YP3*$^-$ chromosomes carry *v*. The *YP2*$^-$ stock was given to us by Postlethwait (see Tamura et al., 1985).

RESULTS

General

The ultrastructure of *Drosophila* vitellogenic ovarian follicles has been reported earlier (King, 1970; Giorgi and Jacob, 1977a,b) and is portrayed diagrammatically in Fig. 1. The onset of vitellogenesis is marked by the appearance of numerous coated pits amongst the microvilli of the oocyte plasma membrane. Upon invagination into the cortical ooplasm, they transform into coated vesicles which in turn give rise to either vesicles or tubules fusing with the forming yolk spheres. During the procedure of yolk deposition, the forming yolk spheres are frequently in close proximity to the Golgi apparatus.

To study the nature of the vesicular traffic between the endocytic pathway and the Golgi apparatus in the cortical ooplasm, the results of four different imposed experimental conditions were examined in this study. These conditions were provided by: (1) follicles made incapable of undergoing vitellogenesis by mutations on regulatory genes, (2) follicles sequestering YPs which differed in the number of wild-type structural genes, (3) follicles modified experimentally by either starvation, refeeding or topical application of a juvenile hormone analogue and (4) follicles cultured in vitrowith or without addition of a juvenile hormone analogue to the culture medium. In all of these instances, the endocytic pathway leading to the formation of yolk spheres was observed through in vivo or in vitro exposure to horseradish peroxidase. At the same time, the Golgi apparatus and the vesicles emerging from it were preferentially labelled by fixation with OZI (see Material and Methods). The extents of the variations induced in the vesicular traffic of the cortical ooplasm in response to either genetical or experimental manipulations were also quantified by morphometric analysis.

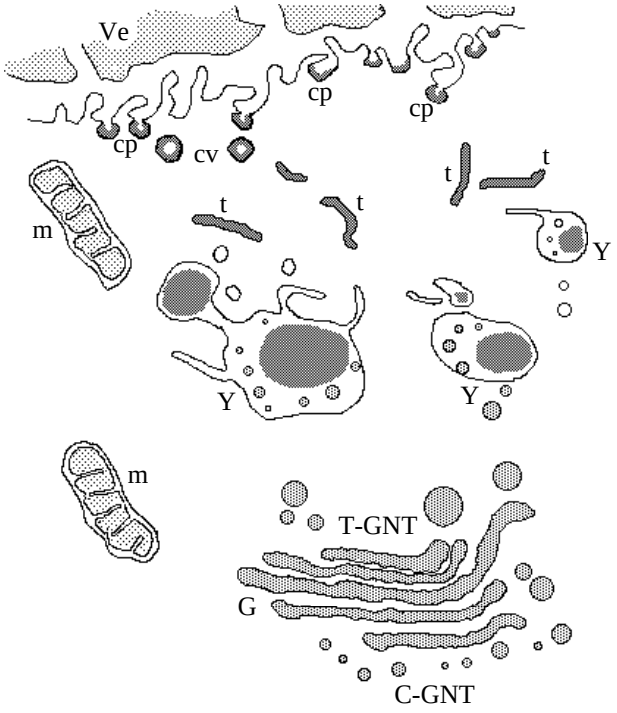

Fig. 1. Schematic drawing showing the cortical ooplasm of a vitellogenic follicle from a wild-type female of *Drosophila melanogaster*. cp, coated pits; cv, coated vesicles; C-GNT, *cis* Golgi network; G, Golgi apparatus; m, mitochondria; t, tubules; T-GNT, *trans* Golgi network; Ve, vitelline envelope; Y, yolk spheres.

Wild-type ovarian follicles

OZI fixation of wild-type oocytes, resulted in labelling of the Golgi apparatus and of a number of vesicles (Fig. 2), but not of the tubules or the coated vesicles of the cortical ooplasm (Fig. 3). OZI-labelled Golgi apparatus appeared either as a long stretch of few flattened cisternae flanked by vesicles on both sides (Fig. 2), or as a cluster of vesicles (Fig. 4), occasionally associated with forming yolk spheres (Fig. 5). These latter structures were also labelled along the asymmetrical superficial layer (Fig. 6). As the yolk spheres enlarged, OZI labelling tended to become regularly scattered along the superficial layer (Fig. 7). In alpha-1 yolk spheres (see King, 1970), OZI labelling was restricted to the region of the superficial layer close to the associated body (Fig. 8). Morphometric analysis of wild-type vitellogenic ovarian follicles showed that 36% of the cortical ooplasm is occupied by the Golgi apparatus, 38% by OZI-labelled vesicles, while 26% is included in the yolk spheres (Fig. 9).

Female-sterile mutations affecting vitellogenesis

To investigate further the nature of the vesicular traffic in the cortical ooplasm, a number of mutant ovarian follicles were examined by electron microscopy after OZI fixation. In *fs(2)A17* follicles, ovarian development is autonomously

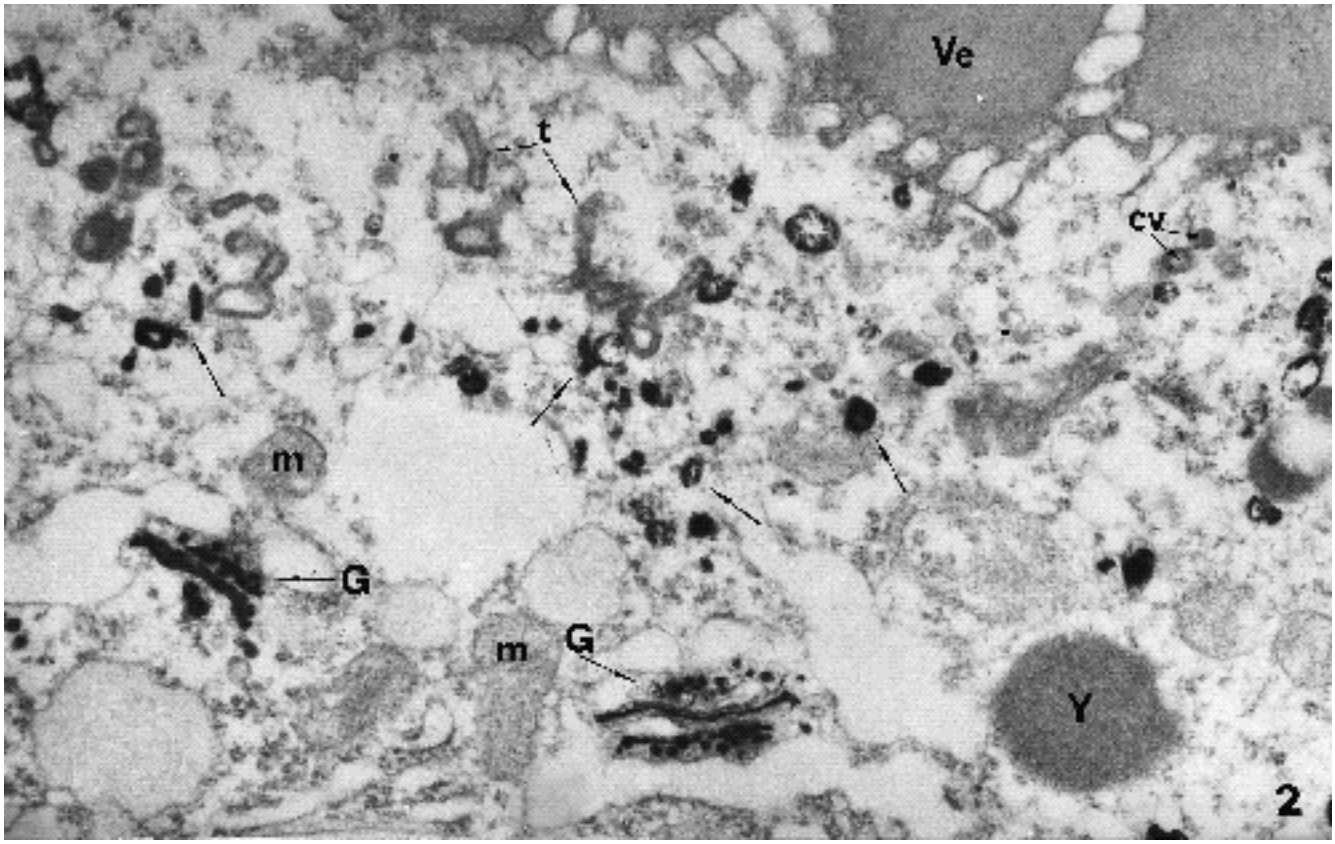

Fig. 2. The cortical ooplasm of an OZI-labelled stage 9 ovarian follicle showing the Golgi apparatus (G) and several OZI-labelled vesicles (arrows). Note that tubules (t) and coated vesicles (cv) are not labelled. Ve, vitelline envelope; Y, yolk sphere; m, mitochondria. ×24,000

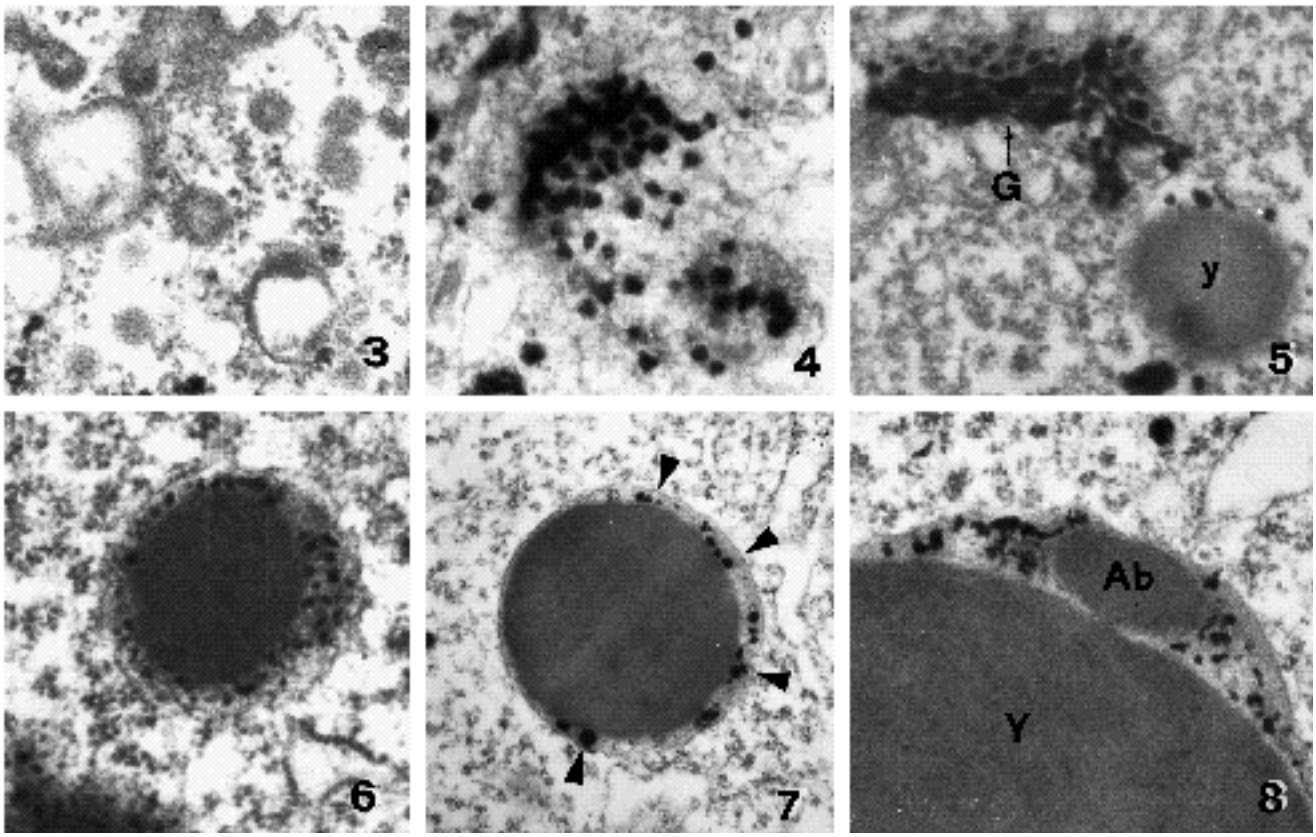

Fig. 3. A high magnification micrograph showing several unlabelled coated vesicles from an OZI-fixed ovarian follicle. ×50,000
Fig. 4. An OZI-labelled Golgi apparatus lying in the cortical ooplasm of a stage 9 wild-type ovarian follicle. ×36,000
Fig. 5. An OZI-labelled Golgi apparatus (G) close to a newly formed yolk sphere (Y) in the cortical ooplasm of a stage 9 wild-type ovarian follicle. ×40,000
Fig. 6. An early yolk sphere containing many OZI-labelled vesicles in the asymmetrical superficial layer. ×40,000
Fig. 7. A yolk sphere with a uniform superficial layer containing several OZI-labelled vesicles (arrowheads). ×18,000
Fig. 8. Part of a fully mature alpha-1 yolk sphere (Y) from a stage 10 ovarian follicle, showing several OZI-labelled vesicles close to the associated body (Ab). ×32,000

restricted to previtellogenesis and the ooplasm contains only dense autophagic organelles. *Drosophila* females homozygous for the gene *apterous*^{4} fail to release physiological amounts of juvenile hormone from the corpora allata and thus, the *apterous*^{4} ovary is blocked in previtellogenesis in a non-autonomous manner (Bownes, 1989). OZI fixation of both these mutant follicles resulted in labelling of only a few peripheral vesicles of the Golgi apparatus (Figs 10 and 11), with no vesicles being labelled in the cortical ooplasm.

L186/Basc is a female-sterile mutant strain which is deficient in YP receptors (Postlethwait, personal communication). *L186/Basc* ovarian follicles complete development even though yolk spheres are not formed. Interestingly, the Golgi apparatus in mutant follicles was labelled as in controls, but very few OZI-labelled vesicles were present

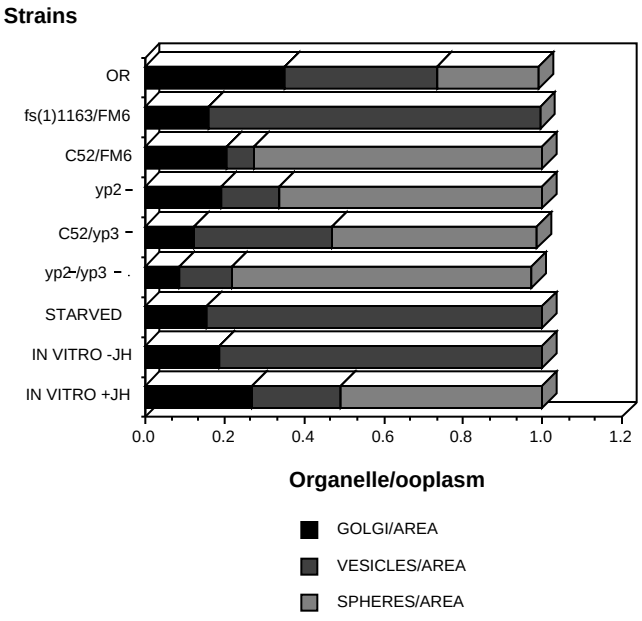

Fig. 9. Histogram showing the relative extension of OZI label in Golgi apparatus, vesicles and yolk spheres of the cortical ooplasm. Labelling was detected in wild-type and mutant *Drosophila* flies and expressed as a percentage of the ooplasm area (organelle/ooplasm). Strains are as specified in Table 1.

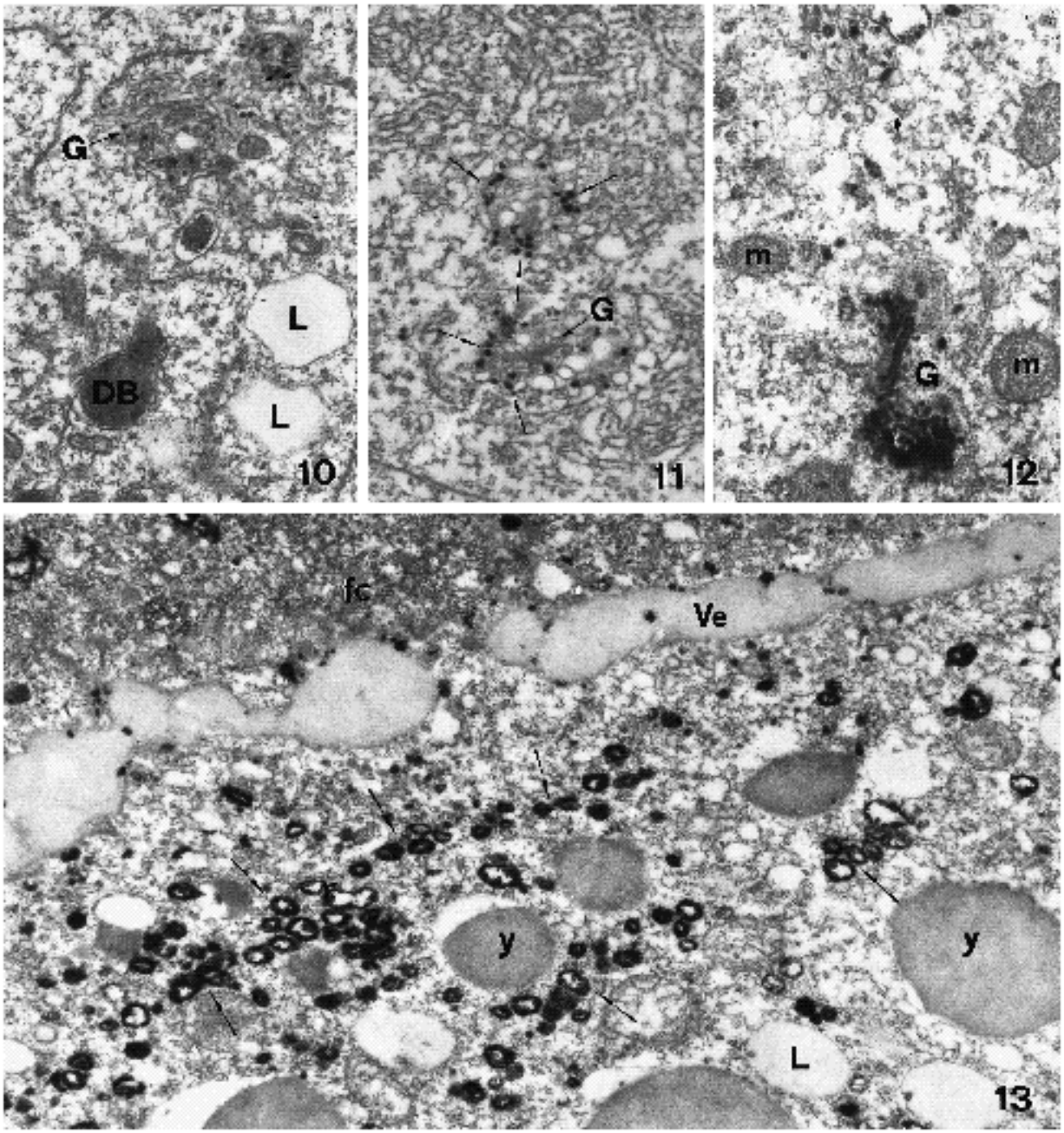

Fig. 10. Low magnification micrograph showing the cortical ooplasm of an OZI-fixed *fs(2)A17* ovarian follicle. G, Golgi apparatus; DB, dense body; L, lipid droplets. ×19,000
Fig. 11. The cortical ooplasm of an OZI-fixed *apterous*^{4} ovarian follicle. Note that only a few marginal vesicles are labelled in the Golgi apparatus (G; arrows). ×24,000
Fig. 12. An OZI-labelled Golgi apparatus (G) from the cortical ooplasm of a *L186* ovarian follicle. m, mitochondria. ×16,000
Fig. 13. The follicle cell/oocyte interface from an OZI-fixed *fs(1)1163* ovarian follicle. Note that vesicles (arrows) but not the yolk spheres (Y) are labelled in the cortical ooplasm. Ve, vitelline envelope; fc follicle cells; L, lipid droplets. ×12,000

in the cortical ooplasm at any developmental stage (Fig. 12).

fs(1)1163 is a female-sterile mutation deficient in YP1 secretion (Bownes and Hodson, 1980; Minoo and Postlethwait, 1985). Yolk deposition in vitellogenic *fs(1)1163* follicles is abnormal, and the resulting embryos incapable of completing development (Giorgi and Postlethwait, 1985). Following OZI fixation, the Golgi apparatus was labelled as in wild-type follicles. However, mutant yolk spheres were either scantly labelled or not labelled at all (Fig. 13).

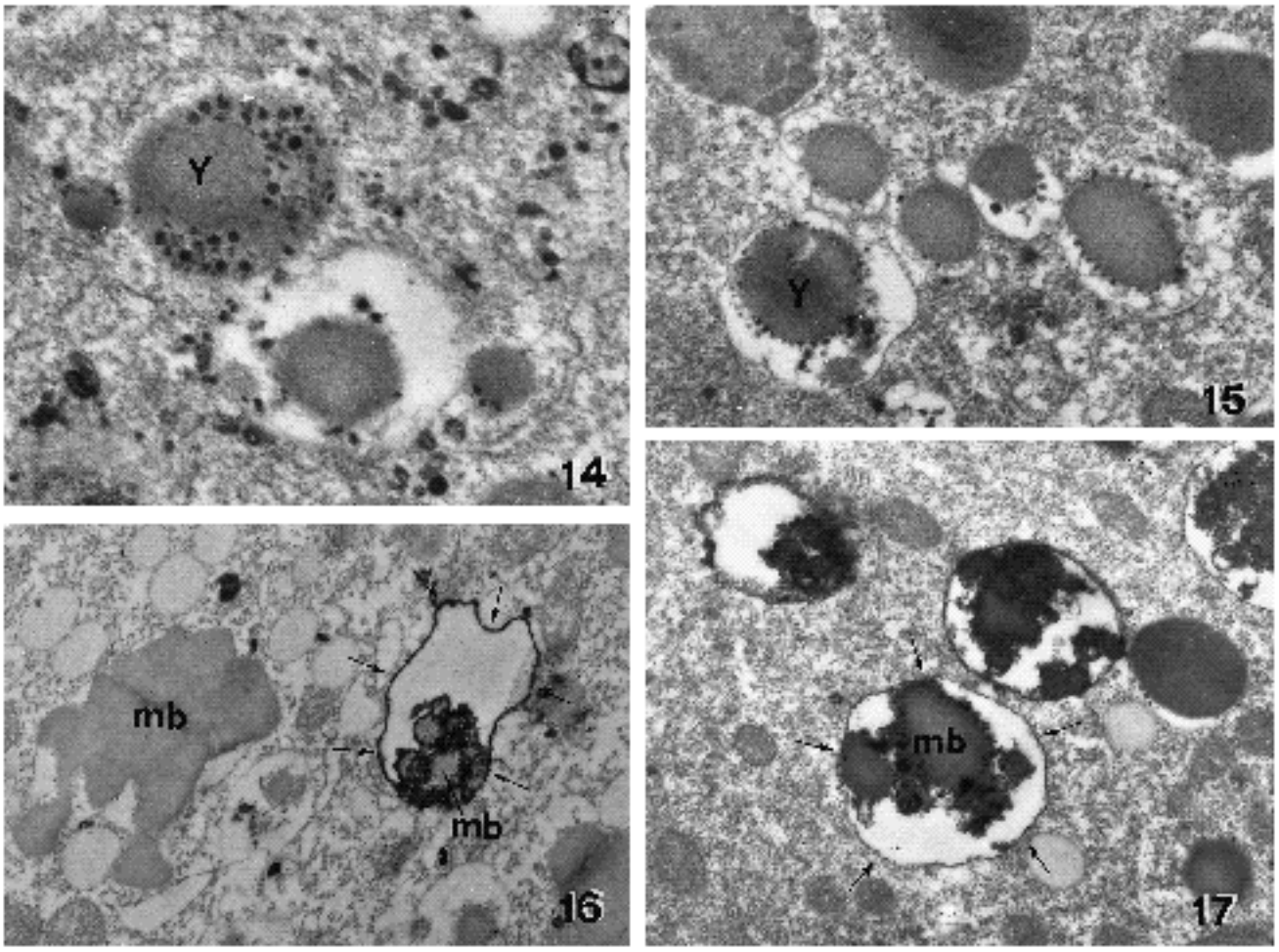

Fig. 14. The cortical ooplasm from a *yp2*⁻ ovarian follicle showing two yolk spheres (Y) with numerous OZI-labelled vesicles therein. Several OZI-labelled vesicles are also present in the surrounding regions. ×30,000
Fig. 15. The cortical ooplasm from an ovarian follicle bearing deletion *C52*. Note that OZI label is restricted to the superficial layer of the yolk spheres (Y) and none is associated with vesicles in the ooplasm. ×12,000
Fig. 16. Abnormal yolk spheres in the cortical ooplasm of a *C52yp3*⁻ ovarian follicle. OZI labels both the main body (mb) and the limiting membrane (arrows). ×12,000
Fig. 17. Abnormal yolk spheres in the cortical ooplasm of a *yp2*⁻*yp3*⁻ ovarian follicle. OZI label extends over the main body (mb) and the limiting membrane (arrows) of the yolk spheres. ×16,000

Conversely, there appeared to be far more OZI-labelled vesicles in *fs(1)1163* cortical ooplasm than in wild-type ovarian follicles at a comparable stage (see histogram in Fig. 9 for morphometric analysis).

Drosophila stocks differing in yolk protein composition

To find out whether the vesicular traffic in the cortical ooplasm depends upon the nature of the protein stored in the yolk spheres, we examined a number of *Drosophila* flies differing in yolk protein composition (see Table 1). In the cortical ooplasm of female flies lacking YP2, there was more OZI label associated with the yolk spheres than with the vesicles nearby (Fig. 14). An even more dramatic situation was found in flies hemizygous for yp1 and yp2 genes in which the OZI label was almost exclusively associated with the yolk spheres (Fig. 15). Both of these conditions were at variance from that found in wild-type follicles where the number OZI-labelled vesicles in the cortical ooplasm is within the range of that associated with the superficial layer of the yolk spheres (Fig. 9).

Female flies bearing chromosome *Df(1)C52YP3⁻/FM6* are hemizygous for all three YPs. Most of their yolk spheres were abnormal (Fig. 16). The main body had an elaborated contour, due perhaps to repetitive fusions of smaller yolk spheres, with no OZI labelling in the superficial layer. However, other yolk spheres were completely labelled, including their limiting membrane. *Drosophila* female flies homozygous for YP2⁻ YP3⁻ genes have only two of the normal yp gene complement. All yolk spheres in these oocytes were characterized by an OZI-positive, irregularly shaped, main body enclosed by a heavily labelled limiting membrane (Fig. 17).

Experimentally modified flies

Vitellogenesis in *Drosophila* is a nutritionally controlled

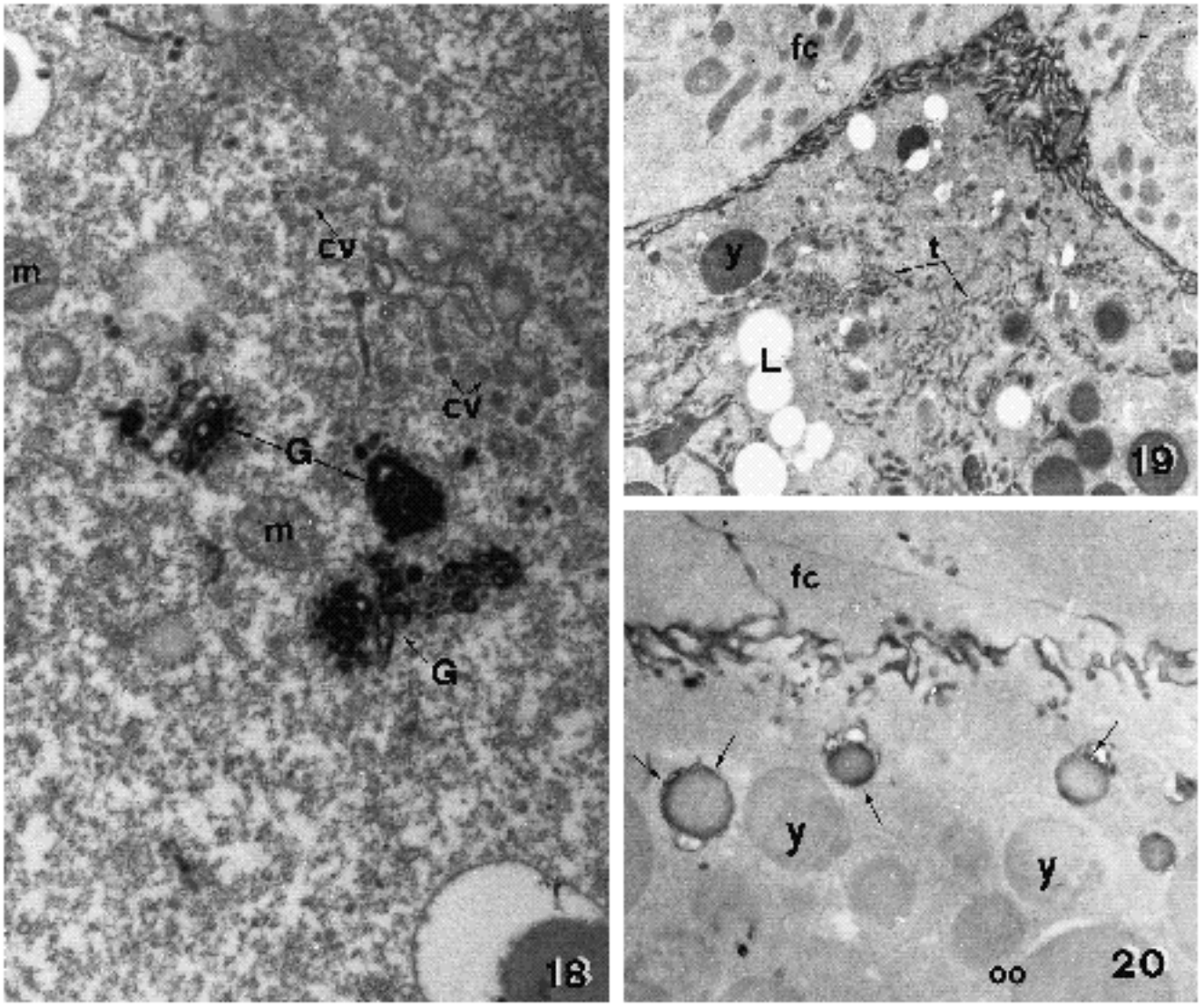

Fig. 18. The cortical ooplasm of a wild-type ovarian follicle fixed with OZI after 24 hours starvation. The Golgi apparatus (G) is labelled as in controls but no OZI-labelled vesicles are present in nearby regions. cv, coated vesicles; m, mitochondria. ×27,000
Fig. 19. The follicle cell/oocyte interface from a wild-type ovarian follicle exposed in vivo to horseradish peroxidase for 1 hour following 24 hour starvation. fc, follicle cell; y, yolk spheres; L, lipid droplets; t, tubular network. ×9,000
Fig. 20. The follicle cell/oocyte interface (fc/oo) from a wild-type ovarian follicle exposed in vivo to horseradish peroxidase for 1 hour following 24 hours starvation and 12 hours refeeding. Unstained section. Arrows point to peroxidase-labelled yolk spheres in the cortical ooplasm. y, unlabelled yolk spheres. ×12,000

process (Bownes and Blair, 1986; Bownes and Reid, 1990). To find out how the vesicular traffic in the cortical ooplasm is affected by nutrition, a number of wild-type female flies were examined for OZI labelling following 24 hours starvation. Fig. 18 shows that under these conditions, the Golgi apparatus was still heavily labelled, but only a few OZI-labelled vesicles were in the cortical ooplasm and none were associated with the yolk spheres. 12 hours of refeeding sufficed to restore the number of OZI-labelled vesicles in association with yolk spheres up to a level comparable to controls. A reversal of yolk sphere labelling was also caused by topical application of a juvenile hormone analogue, ZR-515, to starved female flies (data not shown).

Starved and refed female flies were also examined by electron microscopy following in vivo exposure to horseradish peroxidase. Data showed that peroxidase did not gain access to the yolk spheres of starved female flies, but labelled instead a tubular network in the cortical ooplasm (Fig. 19). 12 hours of refeeding caused the tubular network to disappear from the cortical ooplasm and allowed the tracer to gain access to the superficial layer of newly formed yolk spheres (Fig. 20).

In vitro cultured ovarian follicles

To investigate the hormonal dependence of endocytic uptake in *Drosophila*, a number of ovarian follicles were cultured in Grace's medium and examined for OZI labelling and peroxidase access to the yolk spheres. Vitellogenesis

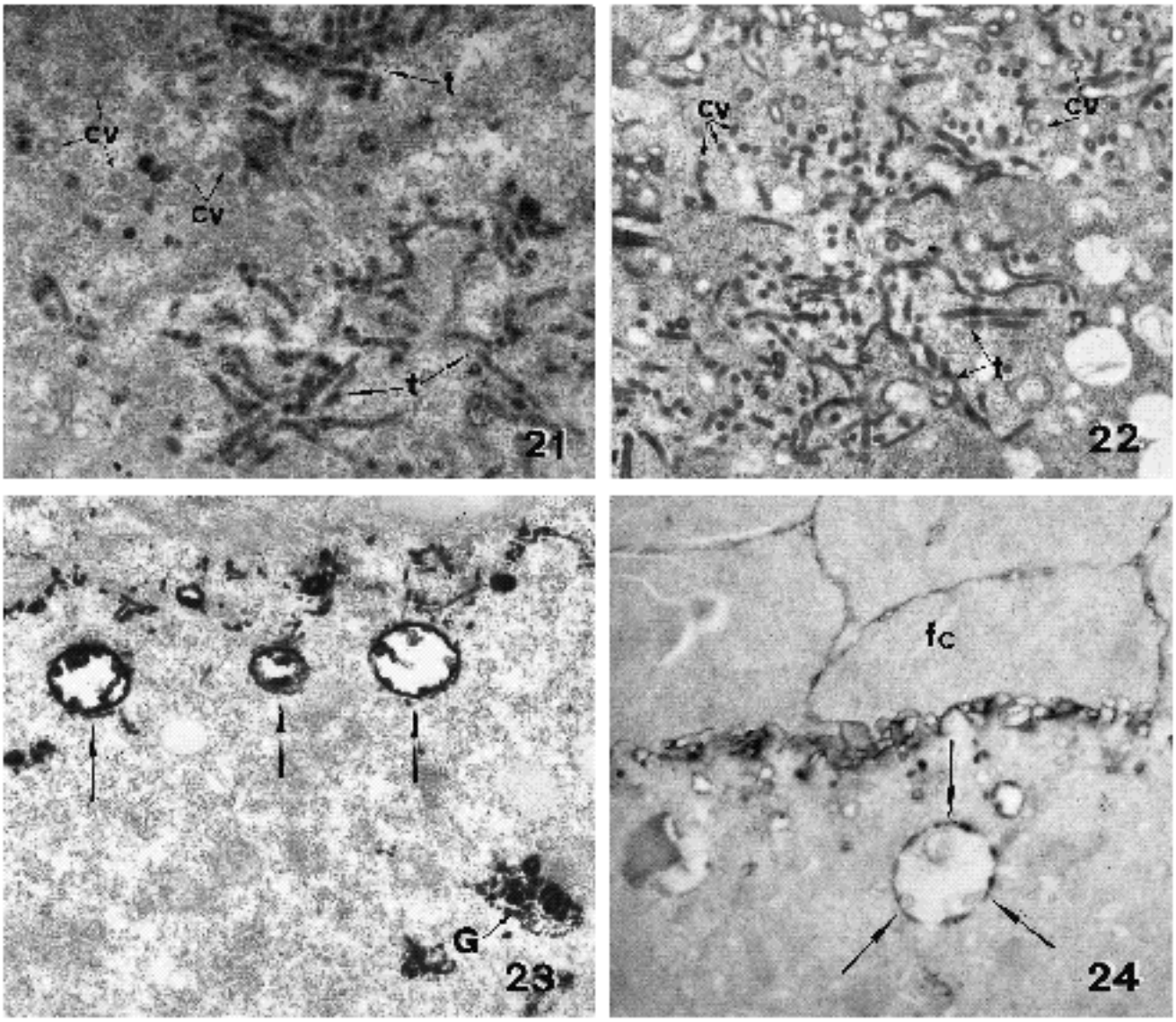

Fig. 21. The cortical ooplasm from a wild-type, OZI-fixed, ovarian follicle cultured in vitrofor 2 hours in Grace's medium. Note the presence of a tubular network (**t**) heavily labelled with OZI. **cv**, coated vesicles. ×15,000

Fig. 22. The cortical ooplasm from a wild-type ovarian follicle cultured in vitrofor 2 hours in Grace's medium in the presence of horseradish peroxidase. The tracer has gained access to both coated vesicles (cv) and tubules (t). ×12,000

Fig. 23. The cortical ooplasm of a wild-type ovarian follicle fixed with OZI 2 hours after in vitro culturing in the presence of ZR-515. Empty-looking yolk spheres (arrows) underneath the oolemma appeared labelled with OZI. The Golgi apparatus (G) is also labelled. ×12,000

Fig. 24. The cortical ooplasm of a wild-type ovarian follicle cultured in vitro for 2 hours in the presence of ZR-515 and horseradish peroxidase. The tracer has gained access to an empty looking yolk sphere (arrows) underneath the oolemma. fc, follicle cells. ×12,000

in *Drosophila* does not proceed normally in vitro, since follicle cell-produced YPs tend to leak out into the culture medium, rather than accumulate in the oocyte. Under these conditions, the cortical ooplasm is consistently characterized by the presence of an extended tubular network (Giorgi, 1979). Most of these anatomising tubules appeared to be labelled following either OZI fixation (Fig. 21) or addition of peroxidase to the culture medium (Fig. 22). Neither OZI nor peroxidase could be shown to label yolk spheres to any major extent in in vitro cultured oocytes. However, while coated pits and vesicles were labelled in follicles exposed to peroxidase, they were never labelled after OZI fixation (compare Figs 21 and 22).

The tubular network was no longer detectable in the cortical ooplasm of follicles exposed in vitro to ZR-515. Under these conditions, OZI label and peroxidase appeared to be associated with large, empty looking, newly formed 'yolk' spheres (Figs 23, 24). In addition, the Golgi apparatus was labelled with OZI as in wild-type ovarian follicles (Fig. 23).

DISCUSSION

The evidence provided in this study demonstrates that the cortical ooplasm of genetically or experimentally modified *Drosophila* female flies can be characterized by three different labelling patterns. The first includes *A17* and *apterous* ^{4} oocytes with no or subliminal labelling of the Golgi apparatus and of any vesicles in close proximity. The second includes starved, cultured, *fs(1)1163* and *Df(1)C52yp3*$^{-}$*/FM6* oocytes, with an excess of vesicles in the ooplasm, and no labelling of the yolk spheres. The third and final labelling pattern includes *Df(1)C52/FM6* and *YP2*$^{-}$ oocytes in which excess vesicles are inside the yolk spheres. The most likely interpretation of these results is that OZI fixation allows visualization of the vesicular traffic between the Golgi apparatus and the yolk spheres. The validity of this interpretation rests upon several observations. OZI fixation is known to be specific for labelling the Golgi apparatus (Maillet, 1963; Niebauer et al., 1969) and the vesicles emerging from it (Giorgi et al., 1976; Matsuura et al., 1976). In addition, the Golgi apparatus has been shown to manifest a clear propensity to give rise to either vesicles or tubules that extend from the *trans* Golgi network (Cleutt and Brown, 1991) to early endosomes (Wood et al., 1991). These findings are all consistent with the generally accepted view that materials destined for the plasma membrane for secretion and/or to the lysosomes for storage are being processed and sorted in the *trans* Golgi network (Griffiths and Simons, 1986; Pfeffer and Rothman, 1987).

The different labelling patterns observed in this study, resulting from either genetic or experimental manipulations, are likely to depend upon variations in the extent to which vesicles are being transferred to the yolk spheres. For instance, if more vesicles are labelled in the cortical ooplasm, as in the case of *fs(1)1163* oocytes, then this is likely to be due to arrest of vesicular transfer to the yolk spheres. On the other hand, follicles bearing deletion *C52* have far more vesicles within the yolk spheres than in the ooplasm. This suggests that vesicular targeting to the yolk spheres in these follicles may have occurred at a normal pace, but YP processing in the superficial layer may have been delayed as compared to controls.

As to the factors controlling vesicular traffic in *Drosophila* oocytes, two potential sites should be considered. While vesicle emergence from the Golgi apparatus may be controlled autonomously (that is by the genetic make up of the oocyte itself), vesicle inclusion into the yolk spheres may depend upon the stability of the internalized YP or the availability of juvenile hormone (Raikhel and Lea, 1985). For instance, incorporation of YPs with fewer than four polypeptides, as in *Df(1)C52yp3*$^{-}$*/FM6* and *yp2*$^{-}$ *yp3*$^{-}$, leads to the appearance of abnormal yolk spheres undergoing degradation. This suggests that incorporation of YPs that are quantitatively deficient in polypeptide composition (Giorgi and Postlethwait, 1985) or lack essential information in their amino acid sequences (Yan and Postlethwait, 1990) causes the yolk spheres to behave as true lysosomes. This interpretation is consistent with earlier observations in in vitro cultured *Xenopus* oocytes showing that vesicular targeting to yolk spheres depends upon the nature and/or the concentration of the ligand to be internalized (Wall and Patel, 1987a). Involvement of secondary lysosomes along the endocytic pathway of *Xenopus* oocytes has also been proved (Wall and Patel, 1987b) and more recently confirmed by ferromagnetic labelling (Richter and Bauer, 1990). Although definite proof about the nature of vesicles involved in *Drosophila* cortical ooplasm during vitellogenesis would rest on the macromolecular resolution of the vesicular traffic, we feel confident to conclude that differential OZI labelling in oocytes differing in yp gene number and composition depends upon the capability of the oocyte to regulate lysosome targeting to the yolk spheres and YP processing.

The cortical ooplasm of follicles experimentally modified by either starvation or in vitro culturing were altered by the appearance of an extended tubular network. Such a structural alteration of the cortical ooplasm has been previously reported in both mutant (Giorgi and Postlethwait, 1985) and in in vitro cultured *Drosophila* follicles (Giorgi, 1979). Tubules are known to constitute an intermediate step along the endocytic pathway leading from the coated vesicles to the yolk spheres (Tsuruhara et al., 1990) and to result from progressive fusion of newly formed endocytic vesicles. Since both OZI and peroxidase gained access to the tubular network, it is quite feasible that this might represent a temporary storage of endocytic vesicles rendered incapable of shuttling to the yolk spheres. As a result, they may end up forming an intermediate compartment to which lysosomes are also contributing (Wood et al., 1991).

The structural modifications induced in the cortical ooplasm by either starvation or in vitro culture could be reversed by refeeding or by ZR-515 administration to the culture medium. This suggests that vesicular transfer to yolk spheres is a hormone-dependent phenomenon. Whether the same mechanism operates in vivo is yet to be demonstrated, since juvenile hormone has been reported not to vary during starvation (Bownes, 1989). In conclusion, the evidence presented here is consistent with a view that targeting of newly endocytosed ligands to lysosomes or storage granules can be controlled both by the nature of the protein internalized and by the hormonal status of the recipient cell. This study may thus pave the way towards understanding how vesicular traffic in vitellogenic follicles affect YP processing and storage in preparation for embryonic development (Bownes et al., 1991).

This work was initiated while F. G. was on leave of absence at the Department of Molecular Biology, University of Edinburgh. This visit was supported by a grant from the European Molecular Biology Organization. Electron micrographs labelled with either OZI or peroxidase were examined for us by Dr Marcello Pagliai, CNR Centre for Soil Science (Pisa), using a Quantimet 570 (Cambridge) image analyzer.

REFERENCES

Belote, J. M., Handler, A. M., Wolfner, M. F., Livak, K. J. and Baker, B. S. (1985). Sex specific regulation of yolk protein gene expression in *Drosophila. Cell* **40,** 339-348.

Bownes, M. and Blair, M. (1986). The effects of sugar diet and hormones on the expression of the *Drosophila* yolk protein genes. *J. Insect Physiol.***32**, 493-501.

Bownes, M. and Hodson, B. A. (1980). Mutant *fs(1)1163* alters yolk protein secretion from the fat body. *Mol. Gen. Genet.* **180**, 411-418.

Bownes, M. (1989). The roles of juvenile hormone, ecdysone and the ovary in the control of *Drosophila* vitellogenesis. *J. Insect Physiol.* **35**, 409-413.

Bownes, M. and Reid, G. (1990). The role of the ovary and nutritional signals in the regulation of fat body yolk protein gene expression in *Drosophila melanogaster. J. Insect Physiol.* **36,** 471- 479.

Bownes, M., Scott, A. and Shirras, A. (1988). Dietary components modulate yolk protein gene transcription in *Drosophila melanogaster*. *Development* **103,** 119 -128

Bownes, M., Lineruth, K. and Mauchline, D. (1991). Egg production and fertility in *Drosophila* depend upon the number of yolk protein gene copies. *Mol. Gen. Genet.* **228**, 324-331.

Cleutt, E. B. and Brown, W. J. (1991). Golgi membranes have a propensity to form tubules and vesicles in the absence of associated coated proteins. *J. Cell Biol.* **115**, 411a

Cummings, M. R. and King, R. C. (1970). The cytology of the vitellogenic stages of oogenesis in *Drosophila melanogaster*. **I.** General staging characteristics. *J. Morph.* **128,** 427-442.

Dhadialla, T. S. and Raikhel, A. S. (1991). Binding of vitellogenin to membranes isolated from mosquito ovaries. *Archs. Insect Biochem. Physiol.* **18,** 55-70.

Ferenz, H. J. (1990). The locust oocyte vitellogenin receptor. Function and characteristics. *Advs. Invert. Reprod.* **5,** 103 -108.

Giorgi, F. (1979). *In vitro* induced pinocytotic activity by a juvenile hormone analogue in oocytes of *Drosophila melanogaster*. *Cell Tissue Res.* **203,** 241-247.

Giorgi, F. (1980). Coated vesicles in the oocyte. In *Coated Vesicles.* (ed. C. A. Ockleford and H. Whyte).pp. 135-177. Cambridge University Press.

Giorgi, F. and Jacob, J. (1977a). Recent findings on oogenesis of *Drosophila melanogaster*. I. Ultrastructural observations on the developing ooplasm. *J. Embryol. Exp. Morph.* **38**, 115 -124.

Giorgi, F. and Jacob, J. (1977b). Recent findings on oogenesis of *Drosophila melanogaster.* II. Further evidence on the origin of yolk platelets. *J. Embryol. Exp. Morph.* **38,** 125 -138.

Giorgi, F. and Postlethwait, J. H. (1985). Yolk polypeptide secretion and vitellin membrane deposition in a female sterile *Drosophila* mutant. *Dev. Genetics.* **6**, 135-150.

Giorgi, F., Bucci, S. and Ragghianti, M. (1976). Osmium zinc iodide staining of Golgi elements in oocytes of *Triturus cristatus. Cell Tissue Res.* **172,** 121-131.

Griffiths, G. and Simons, K. (1986). The trans Golgi network: sorting at the exit site of the Golgi complex. *Science* **234,** 438-442.

King, R. C. (1970). Ovarian development in Drosophila melanogaster. New York: Academic Press.

Koenig, R., Nordin, J. H., Gochoco, C. H. and Kunkel, J. G. (1988). Studies on ligand recognition by vitellogenin receptors in follicle membrane preparations of the german cockroach *Blattella germanica*. *Insect Biochem.* **18,** 395-404.

Mahowald, A. P. and Kambysellis, M. P. (1980). Oogenesis. In *Genetics and Biology of* Drosophila**,** 2d, (ed. T. Ashburner and A. Wright) pp. 141-224.

Maillet, M. (1963). Le réactive au tétraoxyde d'osmium-iodure du zinc. *Z. Zellforsch.Mikrosk. Anat.* **70,** 397-406.

Matsuura, T., Ybata, Y. and Sano, Y. (1976). The zinc iodide osmium tetroxyde reaction on nerve endings in the median eminence of the rat under normal and experimental conditions. *Cell Tissue Res.* **173**, 279-286.

Minoo, P. and Postlethwait, J. H. (1985). Processing and secretion of a mutant yolk polypeptide in *Drosophila*. *Biochem. Genet.* **23**, 913-932.

Niebauer, G., Krawczyk, W. S., Kidd, R. L. and Wilgram, G. F. (1969). Osmium zinc iodide reactive sites in the epidermal Langerhans cell. *J. Cell Biol.* **43**, 80-89.

Pfeffer, S. R. and Rothman, J. E. (1987). Biosynthetic transport and sorting by the endoplasmic reticulum and golgi. *Ann. Rev. Biochem.* **56,** 829-852.

Postlethwait, J. H. and Giorgi, F. (1985). Vitellogenesis in insects. In *Developmental Biology: A comprehensive Synthesis.* (ed. L. W. Browder). **Vol. 1**, pp. 85 -126. New York: Plenum Press.

Raikhel, A. S. (1984). The accumulative pathway of vitellogenin in the mosquito oocyte: a high resolution immuno- and cytochemical study. *J. Ultrastruct. Res.* **87,** 285-302.

Raikhel, A. S. and Dhadialla, T. S. (1992). Accumulation of yolk proteins in insect oocytes. *Annu. Rev. Entomol.* **37**, 217-251.

Raikhel, A. S. and Lea, A. O. (1985). Hormone-mediated formation of the endocytic complex in mosquito oocytes. *Gen. Comp. Endocrinol.* **57,** 422-423.

Raikhel, A. S. and Lea, A. O. (1986). Internalized proteins directed into accumulative compartments of mosquito oocytes by the specific ligand, vitellogenin. *Tissue and Cell* **18,** 559-574.

Richter, H.-P. and Bauer, A. (1990). Ferromagnetic isolation of endosomes involved in vitellogenin transfer into *Xenopus* oocytes. *Eur. J. Cell Biol.* **51,** 53-63.

Roth, T. F. and Porter, K. R. (1964). Yolk protein uptake in the oocyte of the mosquito *Aedes aegypti*. *J. Cell Biol.* **20**, 313-332.

Stoorvogel, W., Strous, G. J., Geuze, H. J., Oorschot, V. and Schwartz, A. L. (1991). Late endosomes derived from early endosomes by maturation. *Cell* **65,** 417-427.

Tamura, T., Kunert, C. and Postlethwait, J. H. (1985). Sex- and cell-specific regulation of yolk polypeptide genes introduced by P-element-mediated gene transfer. *Proc. Natl. Acad. Sci. USA* **82,** 7000-7004.

Tsuruhara, T., Koening, J. H. and Ikeda, K. (1990). Synchronized endocytosis studied in the oocyte of a temperature-sensitive mutant of *Drosophila melanogaster*. *Cell Tissue Res.* **259,** 199-207.

Wall, D. A. and Meleka, I. (1985). An unusual lysosome compartment involved in vitellogenin endocytosis by *Xenopus* oocytes. *J. Cell Biol.* **101,** 1651-1664.

Wall, D. A. and Patel, S. (1987a). The intracellular fate of vitellogenin in *Xenopus* oocytes is determined by its extracellular concentration during endocytosis. *J. Biol. Chem.* **262,** 14779-14789.

Wall, D. A. and Patel, S. (1987b). Multivesicular bodies play a key role in vitellogenin endocytosis by *Xenopus* oocytes. *Dev. Biol.* **119,** 275-289.

Wood, S. A., Park, J. E. and Brown, W. J. (1991). Brefeldin A causes a microtubule mediated fusion of the *trans* Golgi network and early endosomes. *Cell* **67**, 591-600.

Yan, Y.-L. and Postlethwait, J. H. (1990). Vitellogenesis in *Drosophila*: Sequestration of a yolk polypeptide/invertase fusion protein into developing oocytes. *Dev. Biol.* **140**, 281-290.

(Accepted 1 October 1992)