Female sterile (1) yolkless: A Recessive Female Sterile Mutation in *Drosophila melanogaster* with Depressed Numbers of Coated Pits and Coated Vesicles within the Developing Oocytes

Patrick J. DiMario* and Anthony P. Mahowald

Department of Developmental Genetics and Anatomy, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106

Abstract. Ultrastructural analysis of developing oocytes produced by the recessive female sterile mutant, *yolkless* (*yl*), in *Drosophila melanogaster* shows that yl^+ gene activity is necessary for coated pit and coated vesicle formation within these oocytes. 29 alleles of the mutation are known to exist, and they fall either within a strongly affected class or a weakly affected class. Analysis of oocytes produced by females homozygous for the strongly affected class of alleles shows a >90% reduction in the numbers of coated pits and coated vesicles. These oocytes have very little proteinaceous yolk, and the females accumulate vitellogenin (the yolk protein precursor) within their hemolymph. Moreover, females homozygous or hemizygous for a given strong allele produce mature oocytes that are flaccid. Alternatively, females homozygous or hemizygous for weak alleles produce yolk-filled oocytes, but the number of coated pits and coated vesicles within these oocytes is 50% of that found in the oocytes of wild-type females. Despite the presence of yolk within these oocytes, females homozygous for weak yl^- alleles remain sterile, and their mature oviposited eggs collapse with time.

DEVELOPING oocytes of *Drosophila melanogaster* possess large numbers of coated pits within their plasma membranes as well as coated vesicles and anastomosing tubules within their cortices. These subcellular organelles are believed necessary for the endocytosis of vitellogenin, the proteinaceous yolk precursor (Mahowald, 1972). Morphologically similar clathrin-coated organelles are characteristic for receptor-mediated endocytosis of vitellogenin in mosquito (Roth and Porter, 1964) and in chicken oocytes (Roth et al., 1976), or wherever receptor-mediated endocytosis occurs (Goldstein et al., 1979, 1985).

The analysis of mutations affecting receptor-mediated endocytosis should be an excellent approach to increase our understanding of the unique features of this basic cellular process. For instance, much of our understanding of receptor-mediated endocytosis derives from studies of four classes of mutations affecting the low density lipoprotein (LDL) receptor (i.e., Davis et al., 1986; recent review, Goldstein et al., 1985). In addition to the mutations affecting receptors, several mutations have been recovered that affect other aspects of receptor-mediated endocytosis. For example, the endocytic pathway is pleiotropically interrupted in mutant Chinese hamster ovary (CHO) cells that fail to acidify their intracellular vesicles (Klausner et al., 1983; Robbins et al., 1984).

In *Drosophila*, two mutations have been suggested to be involved with endocytosis. First, the temperature-sensitive mutation, *shibere*, is believed to interrupt the endocytic pathway at the coated pit–to–coated vesicle transition (Kosaka and Ikeda, 1983a) in the presynaptic terminals of neuromuscular, sensory, and central nervous system neurons (Kosaka and Ikeda, 1983b). As a result, there are supernumerary coated pits and large cisternae-like involutions of the plasma membranes, with concomitant depletions of coated vesicles. Second, Waring et al. (1983) described an X-chromosome–linked female sterile mutation that produced defective chorions (outer egg shell coverings) and had very little yolk in the oocytes. The genetic defect of this latter mutation has been shown by pole cell transplantations (Waring et al., 1983) and the X-ray–induced mitotic recombination technique (Perrimon et al., 1986) to be dependent on the germ line and not the somatic tissues.

We have undertaken an extensive genetic and cellular analysis of the defect in yolk metabolism in this latter *Drosophila* mutant, which we call *female sterile* (*1*) *yolkless* (also referred to as *yolkless* or simply yl^-). The mutation is recessive; homozygous and hemizygous females are sterile whereas heterozygous females and hemizygous males are fertile. Our results show that the germ line–specific *yolkless* gene affects the number of coated organelles within the oocyte cortex and consequently the endocytosis of vitellogenin.

Materials and Methods

Fly Stocks

Stocks were kept at 25°C on standard *Drosophila* agar-cornmeal-molasses-

P. J. DiMario's present address is Carnegie Institution of Washington, Department of Embryology, 115 W. University Parkway, Baltimore, MD 21210.

Table I. Alleles of fs(1)yolkless

Source	Allele	Strong (S) or weak (W) phenotypes	Oocyte yolk, by light microscopy (wild type=+++*)	Hemolymph vitellogenin (wild type=+‡)
Gans	148	W	++	++
	305	W	+++	+
	ICR756	S	+	++
	1061	W	++	++
	1081	W	+++	+
	1130	W	+++	++
	K184	S	+	++
	K294	W	++	++
	K621	S	+	+++
Mohler	11-73	ND§	ND	ND
	11-380	S	+	+++
	11-432	S	+	++
	12-1259	S	ND	+++
	12-2252	S	ND	++
	12-5004	S	+	++
	12-5262	S	+	++
	14-465	S	+	++
	S5	S	+	+++
	S205	S	+	+++
	L-186	S	+	+++
	L-193	S	+	+++
	L-196	S	+	+++
	L-211	S	+	+++
Engstrom	262b	S	+	+++
	1922	S	++	++
Degelmann	1976	ND	ND	ND
Waring‖	29	S	+	+++
	117	–	–	–
	445	–	–	–

* Small amounts of proteinaceous yolk (+) are observed by light microscopy in the strong alleles (see Fig. 1 *b*) Intermediate levels of yolk (i.e., Fig. 1 *d*) are designated ++, and normal wild-type levels (Fig. 1, *a* and *c*) are designated +++.

‡ Wild-type levels of hemolymph vitellogenin (+) are shown in Fig. 2 *a*, intermediate levels (++) in Fig. 2, *d–f*, and exceptionally high levels (+++) in Fig. 2, *b* and *c*.

§ Not yet determined.

‖ Waring et al. (1983).

yeast medium. Recessive X-chromosome–linked female sterile mutations were balanced with the multiply inverted X-chromosomes *FM3, FM6, FM7, FM7c*, the *M5* chromosome, or *FMO*. Derivations of the *FM7* and the *FM7c* chromosomes are described by Merriam (1969) and Merriam and Duffy (1972), respectively. The balancer chromosome, *FMO*, is *Ins(l)sc*^{8}, *dl-49*, containing the markers y^{3ld}, *B*, *f*, *v*, m^{2}, and *w* (D. Mohler, personal communication). Description of these markers is provided by Lindsley and Grell (1968). Wild-type flies were the Oregon R, P2 strain of *Drosophila melanogaster*, reared in mass culture population cages according to published procedures (Travaglini and Tartof, 1972). Individual flies were manipulated under CO_2 gas anesthesia.

Complementation Tests and Deficiency Mapping

Table I lists all known mutations of the *yolkless* gene. An X-chromosome–linked female sterile mutation was considered allelic and a member of the *yolkless* complementation group if females transheterozygous for a known *yolkless* allele and the female sterile mutation under consideration failed to produce viable eggs. In this manner, *fs(l)262b* failed to complement *fs(l)1922* (Engstrom complementation group) and members of both the Gans complementation group (Gans et al., 1975; Komitoupoulou et al., 1983) and the Mohler complementation group (Mohler, 1977; Mohler and Carrol, 1984). Similarly, *fs(l)1922* failed to complement all members of the Gans group (N. Perrimon, personal communication) and *fs(l)29* of the Waring group (G. Waring, personal communication). These tests, however, do not eliminate the possibility that any of the mutations listed in Table I may also produce *cis*-acting dominant effects on yl^{+} gene expression.

yolkless has been mapped between the 12E1 and 12F1 region of the X-chromosome using *Df(l)g*^{1} (*garnet*^{lethal}) and *Df(l)KA9* (Waring et al., 1983). We have independently mapped yl^{262b} to the same region using the same deficiencies. (Females carrying a wild-type chromosome and either *Df(l)g*^{1} or *Df(l)KA9* are viable and fertile.)

Isolation of Hemolymph Proteins and Gel Electrophoresis

Hemolymph proteins were prepared by opening the abdomens of four females in a total of 40 μl of Ephrussi and Beadle's Ringer solution (Ephrussi and Beadle, 1936), taking care not to damage the ovaries or break the gut and crop. Hemolymph proteins were precipitated with cold 10% trichloroacetic acid (TCA), pelleted at 12,000 *g*, washed twice with cold (−20°C) 100% acetone, and finally air-dried. The protein pellets were then dissolved in 40 μl of SDS lysis buffer. This SDS lysis buffer and the SDS-PAGE system were that of Laemmli (1970). The three *Drosophila* vitellogenin polypeptides, VG1, VG2, and VG3 (respective mol wt of 45,000, 44,000, and 43,000), have been previously identified (Warren and Mahowald, 1979; Brennan and Mahowald, 1982). Immunoprecipitation of radiolabeled vitellogenin with antiserum directed against the oocyte yolk proteins (Warren and Mahowald, 1979) identifies the three vitellogenin proteins in Fig. 2 *g* (q.v.). Initial quantifications of hemolymph vitellogenin from the various *yolkless* alleles (Table I) were accomplished by scanning polyacrylamide gels using wild-type vitellogenin levels for normalization. However, visual inspections of gels similar to those shown in Fig. 2 were subsequently used for Table I. The quantifications in Table I, therefore, are not to be taken as absolute measurements of hemolymph vitellogenin, but as an index for classifying the alleles as either strong or weak.

Light Microscopy

Whole ovaries were fixed in a trialdehyde fixative (Kalt and Tandler, 1971), dehydrated in a graded ethanol series, and embedded in JB-4 plastic (Polysciences, Inc., Warrington, PA). Sections of 2 μm were cut with a dry glass knife and individually placed with forceps onto a drop of 0.5% ammonium hydroxide on subbed slides (Atherton and Gall, 1972). The liquid was evaporated with a hot plate, and the sections were stained with 0.1% Fast Green dissolved in 0.1 M HCl.

Electron Microscopy

For standard transmission electron microscopy (TEM), *Drosophila* ovaries or brain tissues were dissected directly into 2% glutaraldehyde in 0.1 M PBS, pH 6.8. The tissues were fixed as previously described (Mahowald et al., 1979) and embedded at room temperature in Spurr's medium (1969) with propylene oxide as the transitional solvent.

For immunoelectron microscopy, the ovaries were fixed in 2% glutaraldehyde, washed with 0.2 M sucrose in 0.1 M sodium cacodylate buffer, pH 7.4, dehydrated in graded dimethylformamide, and then infiltrated with Lowicryl plastic (Polysciences, Inc.) with dimethylformamide as the transitional solvent (Altman et al., 1983). The Lowicryl plastic was polymerized with two Sylvania 15-W Blacklight Blue bulbs set 10 cm above the closed Beem capsules.

Lowicryl-embedded ovarian thin sections were mounted on naked nickel grids and treated with 1% BSA dissolved in PBS, pH 7.2, over 30 min. The sections were subsequently treated for 2 h with rabbit anti-yolk serum (Warren and Mahowald, 1979) diluted 1:500 in the same PBS buffer containing 1% BSA. The sections were washed with PBS and then probed with affinity purified goat anti-rabbit IgG (Calbiochem-Behring Corp., San Diego, CA) conjugated to colloidal gold. The colloidal gold was previously prepared and conjugated to the goat anti-rabbit IgG as described by Slot and Gueze (1981).

All tissues, whether prepared for standard TEM or immunoelectron microscopy, were stained with uranyl acetate and lead citrate (Frasca and Park, 1965). The tissues were observed with a Zeiss EM109 electron microscope (Carl Zeiss, Inc., Thornwood, NY).

Quantitation of Coated Organelles

Between 60 and 80 stage 10 (King, 1970) egg chambers were hand isolated from three to four females of a given genotype. The egg chambers were pooled for fixation and embedding. From this total, between 6 and 12 sepa-

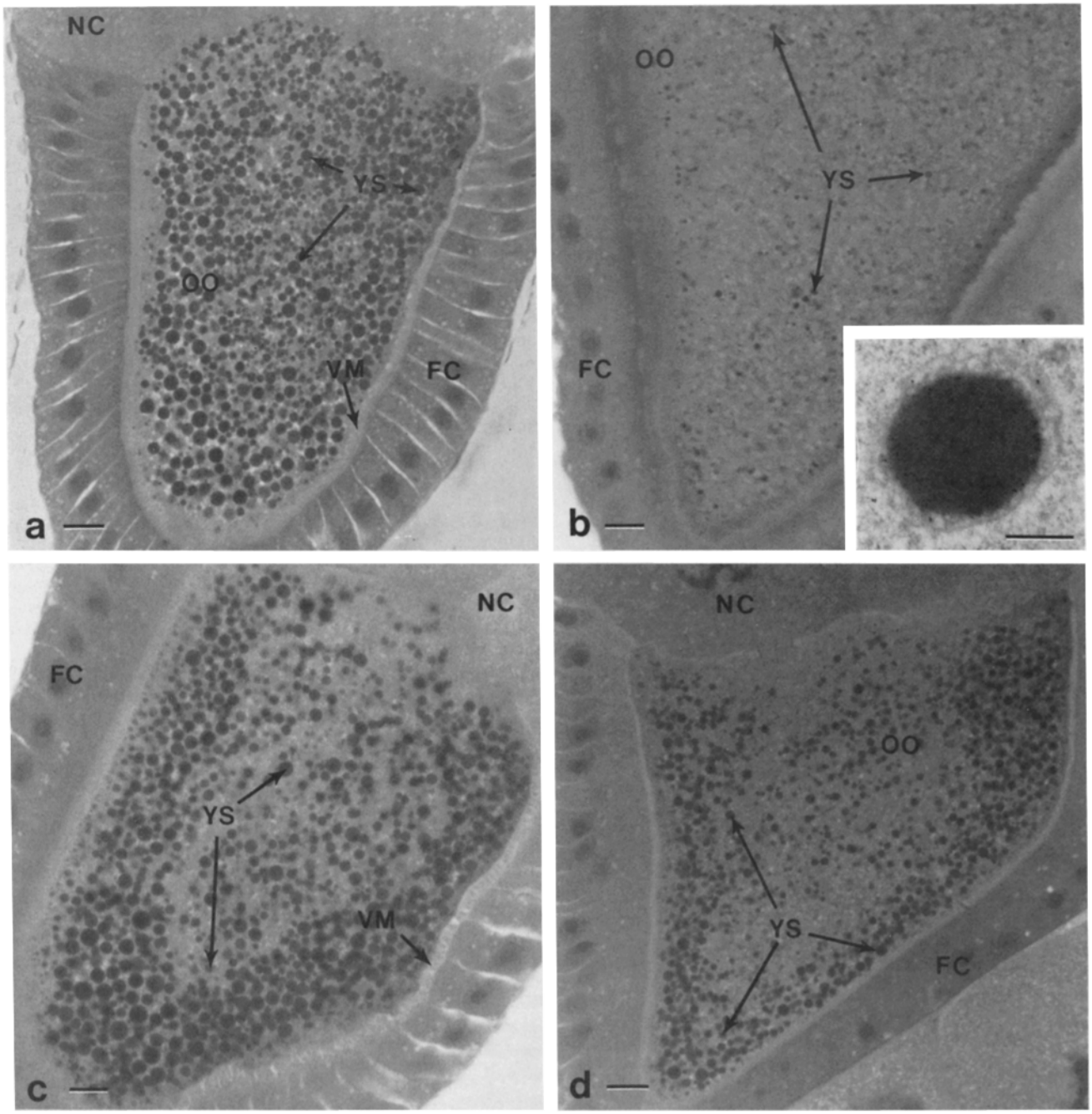

Figure 1. Light micrographs of stage 10b egg chambers obtained from the following sources: (*a*) Homozygous wild-type females. (*b*) Homozygous *262b* females. (*Inset*) Electron micrograph showing the indirect immunocolloidal gold localization of yolk protein within these small spheres. (*c*) Homozygous *1130* females. (*d*) *1130/Df(1)g*^{1} females. The strong alleles, represented here by *262b*, are nearly devoid of yolk spheres. The weak alleles (i.e., *1130*) have yolk. *OO* denotes the oocyte; *NC*, nurse cells; *FC*, follicle cells; *YS*, yolk spheres; *VM*, vitelline membrane which is nearly complete in *c*. Calibration bars, 5 μm. *Inset* calibration bar, 0.3 μm.

rate egg chambers from each genotype were used to quantitate the number of coated structures. The age of the egg chambers was similarly based on the morphology of the still incomplete vitelline membrane. Usually, five micrographs were taken from each egg chamber examined: two micrographs from each of the lateral sides and one from the posterior of the oocyte cortex. The micrographs were taken at ×7,000, and all images were enlarged ×2.12 for a total magnification of ×14,848. A standard unit area of 12 × 3 cm was examined on each micrograph. This is comparable to a cortical area of 8.08 μm in length along the plasma membrane and 2.02 μm in cortical depth. In Table II, *n* refers to the number of separate unit areas used to quantitate the coated structures.

Results

Strong fs(1) yolkless Alleles

The most striking difference at the light microscopic level between wild-type stage 10 oocytes (Fig. 1 *a*) and those produced by females homozygous for the strong *yl*$^{-}$ allele *262b* (Fig. 1 *b*) is the reduction in the number and size of the proteinaceous yolk spheres. The oocytes produced by females homozygous for this or any other strong allele (Table I) are

not completely devoid of yolk (Fig. 1 *b*). Indirect immunogold labeling at the electron microscopic level (*inset* of Fig. 1 *b*) shows that the few immature yolk spheres visible in the *262b/262b* egg chambers do contain yolk protein. No other morphologic abnormalities are readily apparent within the nurse cells or the follicle cells of stage 10 egg chambers at the light microscopic level (but see below for an ultrastructural description of their interfollicular spaces). In addition to the reduced amounts of yolk protein, the mature egg chambers (stages 13 and 14) are found collapsed within the ovaries of strongly affected females.

By light microscopic examinations (data not shown), the hemizygous females, either *262b/Df(1)KA9* or *262b/Df(1)g*^{1} show similar depletions of yolk protein within their oocytes as do the homozygous *262b/262b* females. Mature eggs produced by these deficiency hemizygotes are also found collapsed within their ovaries. Because the reduced amounts of proteinaceous yolk in homozygous and hemizygous females are quite similar, the strong alleles may be lack of function mutations of the *yl* gene.

The hemolymph vitellogenin levels of the strongly affected *262b/262b* and *262b/Df(1)KA9* females are compared with wild-type levels by SDS-PAGE in Fig. 2, lanes *a–c*. It is clear that vitellogenin is synthesized and secreted into the hemolymph of these females, and that there is an inverse relationship between the amount of vitellogenin within the hemolymph and the amount of yolk protein present within their oocytes (Table I). Similar results were obtained for *fs(1)29* by Waring et al. (1983).

Immunoelectron Microscopy of Interfollicular Spaces

Ultrastructural examination of egg chambers produced by females homozygous for the strong allele *262b* (Fig. 3 *a*) as well as other strong alleles (micrographs not shown) shows the presence of electron dense material within the ovarian interfollicular spaces. Immunoelectron microscopy, with rabbit anti-yolk (Warren and Mahowald, 1979) as the first antibody and affinity-purified goat anti-rabbit IgG conjugated to colloidal gold as the second probe, was used to characterize this material. The results (Fig. 3 *a*) show that the material filling the interfollicular spaces contains vitellogenin. The concentration of the colloidal gold in Fig. 3 *a* is nearly as dense as that observed over wild type proteinaceous yolk spheres as shown in Fig. 3 *b*. Thus, the flocculent material filling the interfollicular spaces of strongly affected egg chambers is at least highly enriched for vitellogenin.

The ovaries used for the above immunoelectron microscopic observations were dissected directly into 2% glutaraldehyde, which should preserve any accumulations of vitellogenin within the interfollicular spaces. If the ovaries are first dissected into saline and fixed after 10–20 min, the interfollicular spaces appear free of flocculent vitellogenin (micrographs not shown). This observation suggests that the vitellogenin is not permanently bound within the interfollicular spaces, but instead will diffuse from these spaces (i.e., a decreasing vitellogenin concentration gradient).

In addition to the interfollicular spaces, flocculent vitellogenin has been observed between the completed vitelline membrane (the first egg shell membrane) and the plasma membrane of the oocyte in older egg chambers (micrograph not shown), apparently trapped there by the fusion of vitelline bodies as the vitelline membrane is completed.

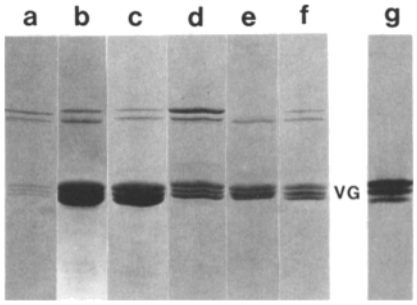

Figure 2. Hemolymph proteins were separated by SDS-PAGE using 10% polyacrylamide gels. Each lane contains the protein from two females. The three vitellogenin polypeptides are designated VG. The two proteins of greater molecular weight than the vitellogenins fluctuate in concentration depending upon the age of the flies. (*a*) Wild type/wild type; (*b*) *262b/262b*; (*c*) *262b/Df(1)KA9*; (*d*) *1130/1130*; (*e*) *1130/Df(1)g*^{1}; (*f*) *1130/262b*; (*g*) immunoprecipitation of radiolabeled vitellogenin with rabbit anti-yolk serum (Warren and Mahowald, 1979).

Weak fs(1)yolkless Alleles

Stage 10 oocytes produced by females homozygous for the weak *yl*$^-$ allele, *1130* (Fig. 1 *c*), as well as other weak alleles (Table I), contain proteinaceous yolk spheres. At least for the *1130* allele, the number and size of these yolk spheres appear normal in many of the examined stage 10 oocytes. But despite the presence of this yolk, the oviposited eggs of *1130/1130* females collapse with time, and the females remain sterile. This collapsed egg phenotype observed for the weak alleles is, however, less severe than that observed for the strong alleles. That is, eggs of strongly affected females (at least for *262b/262b*) collapse in late oogenesis (stages 13–14) within the mother fly, while eggs produced by the weak alleles collapse only after oviposition.

Oocytes produced by hemizygous *1130/Df(1)g*^{1} females (Fig. 1 *d*) and *1130/Df(1)KA9* females (data not shown) also contain proteinaceous yolk spheres. Although the number and size of the yolk spheres in many of the observed *1130/Df(1)g*^{1} oocytes are reduced compared to wild-type levels, the yolk content in some *1130/Df(1)g*^{1} oocytes appears normal. Mature eggs produced by these deficiency hemizygotes also collapse after oviposition, and these females remain sterile.

SDS-PAGE analysis of the hemolymph from *1130/1130* and *1130/Df(1)g*^{1} adult females (Fig. 2, lanes *d* and *e*, respectively) shows that the vitellogenin levels are intermediate between that of wild-type females (Fig. 2, lane *a*) and that of severely affected *262b/262b* females (Fig. 2, lane *b*). Finally, the hemolymph vitellogenin levels in the *trans*-heterozygote, *1130/262b* (Fig. 2, lane *f*), are also intermediate between wild-type and the severely affected *262b/262b* titers. Although not presented, the oocytes of sterile *1130/262b* females also contain yolk protein, suggesting that the *1130* gene must provide sufficient gene activity to cause partial complementation when placed in *trans* to the putative lack of function allele, *262b*.

Quantitation of Coated Pits and Vesicles

Stage 10 egg chambers from wild-type females, homozygous *262b* (strong allele) females, homozygous *1130* (weak allele)

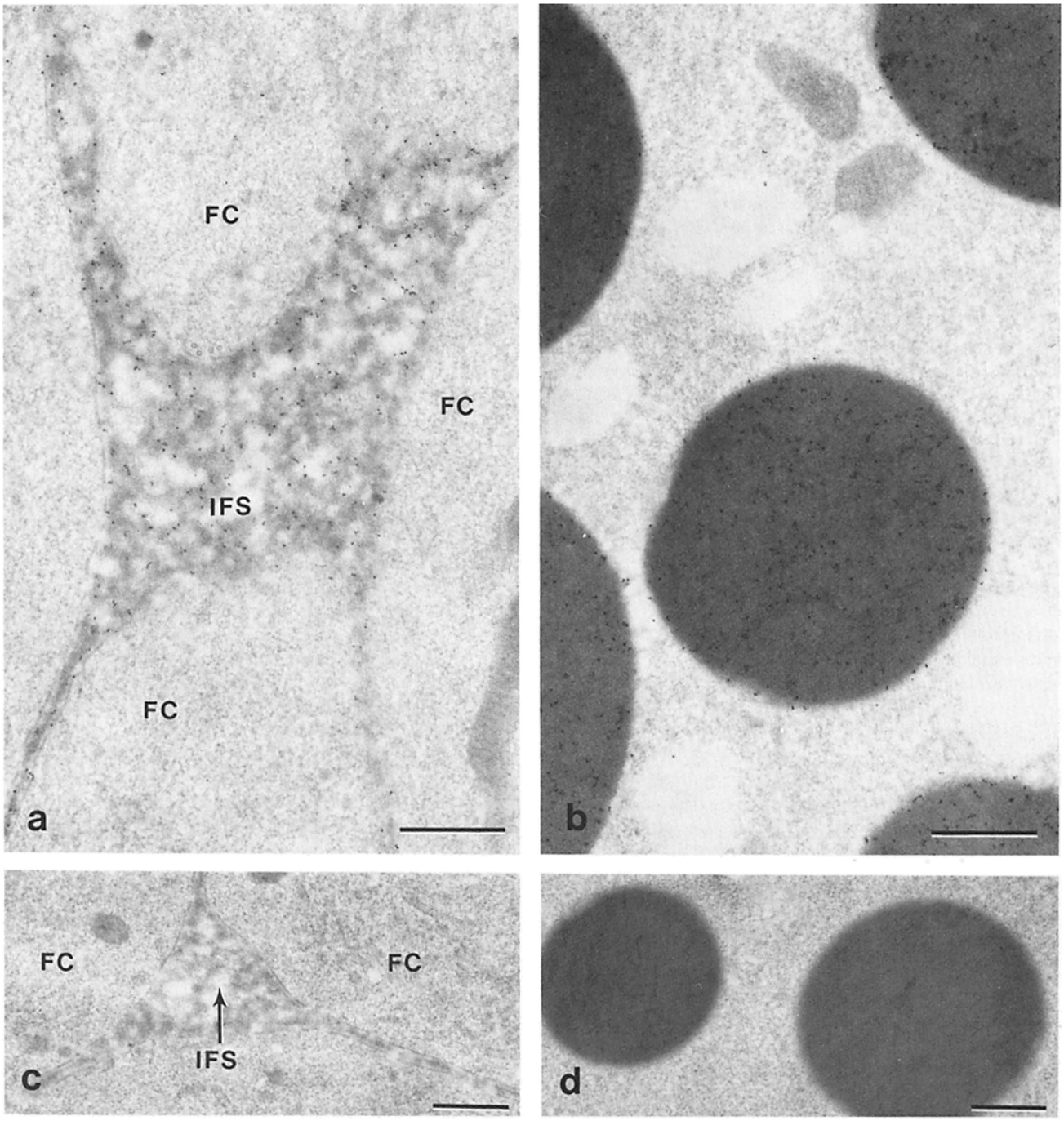

Figure 3. (*a*) Immunoelectron microscopy showing the abnormal accumulation of flocculent material within the interfollicular spaces (*IFS*) of a stage 10 egg chamber produced by a *262b/262b* female. IgG-colloidal gold indirectly localizes vitellogenin within this material. *FC*, follicle cells. (*b*) Wild-type yolk spheres, probed in the same manner as in *a*, show a similar signal intensity. (*c*) Control *262b/262b* grid not treated with primary anti-yolk serum, but probed with gold conjugated second antibody. (*d*) Control wild-type grid treated in the same manner as in *c*. Calibration bars, 0.5 μm.

females, the hemizygous *262b/Df(1)KA9* and *1130/Df(1)KA9* females, and the *trans*-heterozygous *262b/1130* females were used to quantitate the number of coated pits and coated vesicles within the plasma membrane and cortex of their respective oocytes. For each genotype, the number of coated organelles (pits and vesicles) were scored within 30–32 equivalent unit areas of oocyte cortex (see Materials and Methods and Table II).

Stage 10 oocytes produced by *262b/262b* (Fig. 4 *b*) females contain ~9% of the number of coated organelles normally found within wild-type oocytes (Fig. 4 *a*). The few coated vesicles that are present appear to have morphologically normal coats (*inset* of Fig. 4 *b*). Similar, reduced frequencies (~8%) of coated organelles are observed in stage 10 oocytes of the hemizygous female, *262b/Df(1)KA9* (Fig. 4 *c*). Another significant difference between the strongly affected

Table II. Quantitation of Coated Pits and Coated Vesicles

Genotype	Number of coated pits and vesicles per unit area of oocyte cortex	Wild type/ wild type
		%
Wild type/wild type	52.40 SD = 12.3; n = 32	100
262b/262b	4.64 SD = 3.78; n = 31	8.9
262b/Df(1)KA9	4.26 SD = 2.10; n = 30	8.1
1130/1130	26.71 SD = 5.96; n = 31	51
1130/Df(1)KA9	21.80 SD = 6.10; n = 30	42
1130/262b	26.37 SD = 7.70; n = 30	50

oocytes and wild-type oocytes is the almost complete lack of anastomosing tubules characteristic of receptor-mediated endocytosis.

Stage 10 oocytes produced by *1130/1130* females (Fig. 4 *d*) have ~50% of the number of coated organelles per unit area as wild-type oocytes. Like the intermediate hemolymph vitellogenin titers described above for *1130/1130* females, this 50% value for coated organelles in weak allele oocytes is an intermediate phenotype as compared with wild-type (normalized to 100%) and the strong yl^- alleles (9% of wild-type quantities). No attempts were made to quantitate the anastomosing tubules.

Stage 10 oocytes of hemizygous *1130/Df(1)KA9* females and *trans*-heterozygous *1130/262b* females also display an intermediate number of coated organelles (42% and 50%, respectively, Fig. 4, *d* and *e*). Although not quantitated, coated vesicles are also clearly evident in the oocytes of hemizygous *1130/Df(1)g*^{1} females. These latter hemizygous and *trans*-heterozygous examples are significant because they show that the *1130* allele produces an intermediate coated organelle phenotype, in correlation with its intermediate oocyte yolk content and hemolymph vitellogenin levels.

Discussion

We have described an X-chromosome-linked recessive female sterile mutation in *Drosophila melanogaster*, *fs(1)yolkless*, in which there is a reduction in the number of coated pits and coated vesicles in the developing oocytes. Two classes of yl^- alleles exist. Females homozygous for a strong allele produce oocytes that have greatly reduced numbers of oocyte coated pits and coated vesicles. In the case of the extreme *262b* allele, ~9% of the normal wild-type quantities of coated organelles are present. As a probable consequence, these oocytes lack proteinaceous yolk and the females accumulate vitellogenin within their hemolymph. Mature stage 14 oocytes produced by these severely affected females are flaccid probably due, at least in part (see below), to the lack of yolk protein. The females themselves are sterile. Because the deficiency hemizygous *262b/Df(1)KA9* females are indistinguishable from the *262b/262b* females, the *262b* allele may very well be a lack of function allele, at least with regards to the various phenotypes examined.

The second less extreme class of *yolkless* alleles is represented by the *1130* allele. The oocytes of homozygous and hemizygous *1130* females contain approximately half the normal number of coated organelles found in wild-type oocytes of comparable age. As a result, these oocytes contain yolk protein. But vitellogenin still accumulates within the hemolymph of these females, suggesting that their oocytes may be deficient in vitellogenin endocytosis. Because the quantity of coated organelles in *1130/Df(1)KA9* oocytes is the same as in *1130/1130* oocytes, one copy of the *1130* gene appears to be sufficient to establish the same number of coated organelles as two copies of the *1130* gene. Also, inasmuch as the number of coated organelles in the oocytes of *1130/262b* females is similar to that in the oocytes of *1130/Df(1)KA9* females (Fig. 4 *e* and Table II), the *1130* allele must provide the same degree of rescue for the putative lack of function allele, *262b*, as it does for the chromosomal deficiency, *Df(1)KA9*. The product of this single *1130* gene is still defective, though, because these females remain sterile.

The presence or absence of coated pits and coated vesicles within the cortex of the oocytes produced by females of the genotypes in Table II closely corresponds to the presence or near absence of proteinaceous yolk (cf. Table II and Fig. 1). The few coated pits and vesicles present within the oocytes of *262b/262b* and *262b/Df(1)KA9* females may account for the few immature yolk spheres found within these oocytes (e.g., Fig. 1 *b*). In addition, the intermediate number of coated structures in oocytes produced by *1130/1130*, *1130/Df(1)KA9*, and *1130/262b* females is sufficient for the endocytosis of enough vitellogenin to initiate the formation of mature proteinaceous yolk spheres.

The failure of vitellogenin endocytosis is not due to defective vitellogenin polypeptides. Because *yolkless* is germ line dependent (Waring et al., 1983), the failure of vitellogenin endocytosis must be attributed to the germ cells of the egg chambers (i.e., the oocyte and/or its sister nurse cells) rather than to the somatically derived follicle cells surrounding the oocyte, or to vitellogenin, a somatic cell product. If somatic cells homozygous or hemizygous for yl^- were affected, the genetic lesion leading to reduced coated pits and coated vesicles would probably be lethal. Coated vesicles have been observed within the somatic brain tissues of wild-type and *262b/262b* females. The frequencies at which they are found, however, is surprisingly low for both genotypes (data not presented), and no attempts were made to quantitate these coated organelles.

Waring et al. (1983) described several abnormalities in the anterior-dorsal portion of the chorion (the operculum) of eggs produced by *fs(1)29*. These include an underdeveloped chorionic collar, a lack of prominent follicle cell imprint patterns, and morphologic abnormalities in and between the bases of the chorionic appendages that anchor to the operculum. These various chorion abnormalities are probably a result of a cascade of pleiotropic abnormalities. For example, the reduced number of coated organelles in strongly affected oocytes is probably responsible for the lack of yolk protein (as well as the accumulation of vitellogenin in the hemolymph). The lack of yolk protein, in turn, causes a loss of oocyte turgidity. Inasmuch as the follicle cells are normally positioned over the oocyte during choriogenesis, a flaccid oocyte may misposition the follicle cells which then leads to the deformed operculum (Waring et al., 1983). This cascade of phenotypes, starting with the lack of coated organelles, may certainly be true for the strong alleles. But the weak alleles have yolk within their oocytes, and in most cases, we have observed that the mature eggs of weakly affected fe-

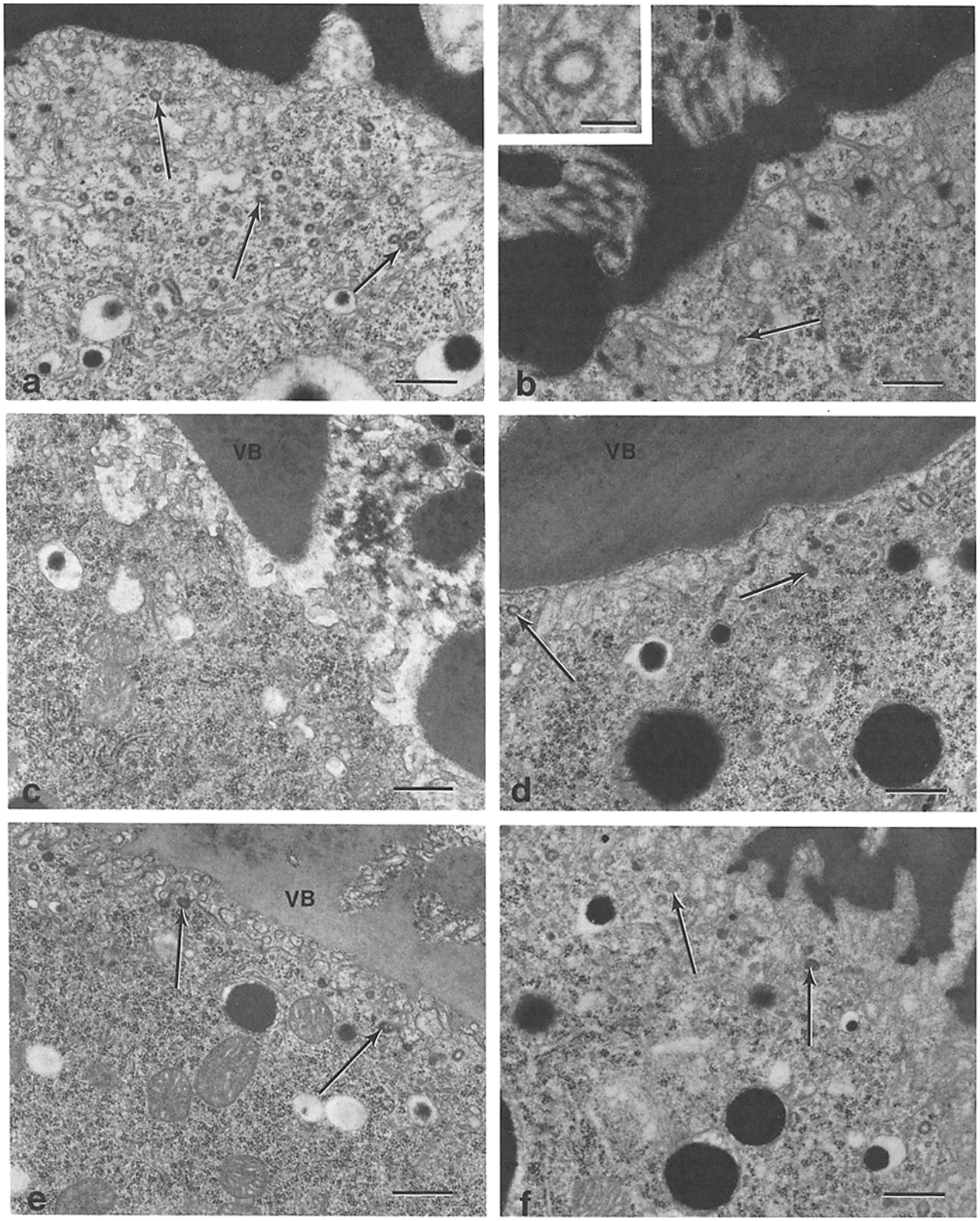

Figure 4. Transmission electron microscopy showing the presence or near absence of coated organelles (*arrows*) within the cortex of wild-type or yl^- stage 10 egg oocytes. (*a*) Wild type/wild type; (*b*) *262b/262b*; (*inset*) coated vesicle with a fairly normal coat; (*c*) *262b/Df(1)KA9*; (*d*) *1130/1130*; (*e*) *1130/Df(1)KA9*; (*f*) *1130/262b*. *VB*, vitelline bodies. Calibration bars, 0.5 μm. *Inset* calibration bar, 0.1 μm.

males collapse only after oviposition. Indeed, our preliminary scanning electron microscope analysis shows that the mature oocytes of homozygous *l130* females have normal opercula.

The actual defect of *yolkless* remains unknown, and any number of possibilities exist. For example, if vitellogenin endocytosis is induced by the developing oocyte's ability to respond to particular hormonal signals (i.e., juvenile hormone; Giorgi, 1979), perhaps the *yolkless* oocytes fail to recognize these signals. Alternatively, if the *Drosophila* oocyte responds to vitellogenin challenge through its receptors (cf. Salisbury et al., 1980), defects in these receptors could then be responsible for the failure of coated pit and coated vesicle formation. Besides the receptors, defects could reside within one of the structural coat proteins such as clathrin or its associative proteins. In that *yl* is germ line dependent, the requirement of the structural gene product would have to be limited to the germ cells.

Finally, a regulatory component of the oocyte cortex could be defective. Such a component may regulate the assembly of clathrin into coats (i.e., the reduced coated organelle phenotype), as well as influence either the osmoregulation or cytoskeleton (i.e., the collapsed egg phenotype). This latter possibility is attractive because the eggs produced by females homozygous for the weak alleles still collapse despite near normal levels of yolk spheres. The female sterility of these weak alleles implies that the near absence of coated organelles and yolk in the oocytes of strongly affected females (e.g., *262b/262b*) may be a pleiotropic abnormality of a defective gene product that is needed for more fundamental processes of oocyte cortex metabolism. For this reason the weak *yolkless* alleles, such as *l130*, may actually be a key to understanding the locus. Future genetic and molecular characterizations of the *yolkless* gene should provide insights about the true gene product and its function.

The authors thank Drs. J. Dawson Mohler, Lee Engstrom, Norbert Perrimon, and Heidi Degelmann for kindly providing female sterile stocks. We especially thank Dr. Norbert Perrimon for his insightful observations and discussions. Portions of this work were carried out in the Dept. of Biology, Indiana University, Bloomington.

This work was supported by National Institutes of Health grant HD 17607.

Received for publication 20 October 1986, and in revised form 10 March 1987.

References

Altman, L. G., B. G. Schneider, and D. S. Papermaster. 1983. Rapid (4 hr) method for embedding tissues in Lowicryl for immunoelectron microscopy. *J. Cell Biol.* 97:309*a*. (Abstr.)

Atherton, D., and J. Gall. 1972. Salivary gland squashes for in situ nucleic acid hybridization. *Drosophila Inform. Serv.* 49:131–133.

Brennan, M. D., and A. P. Mahowald. 1982. Phosphorylation of the vitellogenin polypeptides of *Drosophila melanogaster*. *Insect Biochem.* 12:669–673.

Davis, C. G., M. A. Lehrman, D. W. Russell, R. G. W. Anderson, M. S. Brown, and J. L. Goldstein. 1986. The J.D. mutation in familial hypercholesterolemia: amino acid substitution in cytoplasmic domain impedes internalization of LDL receptors. *Cell.* 45:15–24.

Ephrussi, B., and G. W. Beadle. 1936. A technique of transplantation for *Drosophila*. *Am. Nat.* 70:218–225.

Frasca, J. M., and V. R. Park. 1965. A routine technique for double staining ultrathin sections using uranyl and lead salts. *J. Cell Biol.* 25:157–161.

Gans, M., C. Audit, and M. Masson. 1975. Isolation and characterization of sex-linked female sterile mutants in *Drosophila melanogaster*. *Genetics.* 81:683–704.

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

Goldstein, J. L., R. G. W. Anderson, and M. S. Brown. 1979. Coated pits, coated vesicles, and receptor mediated endocytosis. *Nature (Lond.).* 279: 679–684.

Goldstein, J. L., M. S. Brown, R. G. W. Anderson, D. W. Russel, and W. J. Schneider. 1985. Receptor-mediated endocytosis: concepts emerging from the LDL receptor system. *Annu. Rev. Cell Biol.* 1:1–39.

Kalt, M. R., and B. Tandler. 1971. A study of fixation of early amphibean embryos for electron microscopy. *J. Ultrastruct. Res.* 36:633–645.

King, R. C. 1970. Ovarian Development in *Drosophila melanogaster*. Academic Press, Inc., New York.

Klausner, R. D., G. Ashwell, J. van Renswoude, J. B. Harford, and K. R. Bridges. 1983. Binding of apotransferrin to K562 cells: Explanation of the transferrin cycle. *Proc. Natl. Acad. Sci. USA.* 80:2263–2266.

Komitopoulou, K., M. Gans, L. H. Margaritis, F. C. Kafatos, and M. Masson. 1983. Isolation and characterization of sex-linked female sterile mutants in *Drosophila melanogaster* with special attention to eggshell mutants. *Genetics.* 105:897–920.

Kosaka, T., and K. Ikeda. 1983a. Possible temperature-dependent blockage of synaptic recycling by a single mutation in *Drosophila*. *J. Neurobiol.* 14: 207–225.

Kosaka, T., and K. Ikeda. 1983b. Reversible blockage of membrane retrieval and endocytosis in the garland cell of the temperature-sensitive mutant of *Drosophila melanogaster, shibere*^{ts1}. *J. Cell Biol.* 97:499–507.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of bacteriophage T4. *Nature (Lond.).* 227:680–685.

Lindsley, D. L., and E. H. Grell. 1968. Genetic variations of *Drosophila melanogaster*. *Carnegie Inst. Wash. Publ. No. 627.*

Mahowald, A. P. 1972. Ultrastructural observations on oogenesis in *Drosophila*. *J. Morphol.* 137:29–48.

Mahowald, A. P., J. H. Caulton, and W. J. Gehring. 1979. Ultrastructural studies of oocytes and embryos derived from female flies carrying the *grandchildless* mutation in *Drosophila melanogaster*. *Dev. Biol.* 69:118–132.

Merriam, J. R. 1969. A "new" first chromosome balancer. *Drosophila Inform. Serv.* 44:101.

Merriam, J. R., and C. Duffy. 1972. First multiple seven: now contains $sn^{\times 2}$ for better balancing. *Drosophila Inform. Serv.* 48:43.

Mohler, J. D. 1977. Developmental genetics of the *Drosophila* egg. I. Identification of 50 sex-linked cistrons with maternal effects on embryonic development. *Genetics.* 85:259–272.

Mohler, J. D., and A. Carrol. 1983. Sex-linked female sterile mutations. *Drosophila Inform. Serv.* 60:236–241.

Perrimon, N., D. Mohler, L. Engstrom, and A. P. Mahowald. 1986. X-linked female-sterile loci in *Drosophila melanogaster*. *Genetics.* 113:695–712.

Robbins, A. R., C. Oliver, J. L. Bateman, S. S. Krag, C. J. Galloway, and I. Mellman. 1984. A single mutation in Chinese hamster ovary cells impairs both Golgi and endosomal functions. *J. Cell Biol.* 99:1296–1308.

Roth, T. F., J. A. Cutting, and S. B. Atlas. 1976. Protein transport: A selective membrane mechanism. *J. Supramol. Struct.* 4:527(487)–548(508).

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

Salisbury, J. L., J. S. Condeelis, and P. Satir. 1980. Role of coated vesicles, microfilaments, and calmodulin in receptor-mediated endocytosis by cultured B lymphoblastoid cells. *J. Cell Biol.* 87:132–141.

Slot, J. W., and H. J. Geuze. 1981. Sizing of protein A-colloidal gold probes for immunoelectron microscopy. *J. Cell Biol.* 90:533–536.

Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruc. Res.* 26:31–43.

Travaglini, E. C., and D. Tartof. 1972. Instant *Drosophila*: A method for mass culturing large numbers of *Drosophila*. *Drosophila Inform. Serv.* 48:157.

Waring, G. L., J. P. DiOrio, and S. Hennen. 1983. Isolation of germ line-dependent female-sterile mutation that affects yolk specific sequestration and chorion formation in *Drosophila*. *Dev. Biol.* 100:452–463.

Warren, T. G., and A. P. Mahowald. 1979. Isolation and partial chemical characterization of three major yolk polypeptides from *Drosophila melanogaster*. *Dev. Biol.* 68:130–139.