

Genetically Modified Yolk Proteins Precipitate in the Adult *Drosophila* Fat Body

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Abstract. Ultrastructural and genetic studies were carried out on the fat body of a female sterile mutant *fs(1)1163* to ascertain why yolk protein 1 (YPI) is not secreted from this tissue. Earlier molecular studies demonstrated that (a) normally yolk protein is synthesized in the fat body, secreted into the hemolymph and taken up by the ovary, (b) the *1163* mutation causes a single amino acid substitution in YPI, and (c) females homozygous for the mutation, or heterozygous females raised at 29°C, retain YPI in the fat body. Ultrastructural analysis in this paper shows that the fat body of these females contains masses of electron-dense material deposited in the subbasement membrane space. This subbasement membrane material (SBMM), which

occasionally has a crystalline-like, fibrous component, is found in females whose genotypes include at least one copy of the mutant *1163* gene. These strains include a deletion strain that is hemizygous for the *1163* gene and two strains that are transgenic for the mutant gene. Immunogold studies indicate that SBMM contains yolk protein. We propose that the mutant protein is secreted into the subbasement membrane space, but because of the amino acid substitution in YPI, the oligomers containing YPI condense into SBMM, which cannot penetrate the basement membrane. The similarity of SBMM and deoxyhemoglobin S fibers is discussed.

THE yolk proteins of *Drosophila melanogaster* (YPI, YP2, and YP3), produced by three X-linked genes are synthesized in the fat body (analogous to the vertebrate liver), secreted into the hemolymph, and sequestered in the oocyte by receptor-mediated endocytosis (Bownes and Hames, 1978; Bownes and Hodson, 1980). A second site of synthesis is the ovarian follicle cells (Isaac and Bownes, 1982; Brennan et al., 1982), where the proteins probably enter the interfollicular spaces before endocytosis into the oocyte. The proteins not only provide a nutritional supply, but also bind conjugated hormones needed for embryonic development (Bownes et al., 1988). The genes (*yp1*, *yp2*, and *yp3*), encoding the yolk proteins have proved valuable for studying the sex, tissue, and hormonal regulation of gene expression (see review by Bownes, 1986).

Although YP is synthesized in the fat body, there is no biochemical (Bownes and Hodson, 1980) or cytological evidence (Butterworth and Bodenstern, 1968; Johnson and Butterworth, 1985) to suggest that yolk proteins are normally stored in the tissue. In this paper we analyze a female sterile mutant which we have shown by SDS-PAGE analysis accumulates YPI in the fat body, and that very little of it enters the hemolymph or is sequestered in oocytes (Bownes and Hodson, 1980). The normal YP2 and YP3 proteins are also

accumulated to some degree in the mutant fat body though some of them reach the hemolymph and oocytes. The mutant *fs(1)1163* when homozygous is female sterile at 18 and 20°C; but when heterozygous, it is dominant, female sterile at 29°C. This temperature sensitivity is correlated with the dramatically reduced secretion of YPI and accumulation of YPs 1, 2, and 3 in the fat body (Bownes and Hodson, 1980). The female sterility is unlikely to be caused by the lack of YPI in the eggs but rather seems to be due to the abnormalities in the secretory apparatus of the follicle cells caused by the abnormal YPI protein (Giorgi and Postlethwait, 1985). They concluded that the defective YPI interferes with the deposition of the vitelline membrane which results in eggs that collapse.

The three yolk proteins show many common processing steps and all have a typical leader sequence which is cotranslationally removed. The primary translation products of YPI and YP2 have a molecular mass of 45,000 D, while that of YP3 is ~46,000 D (Bownes and Hames, 1978). The 1-kD leader sequence is removed from each polypeptide, then YPI is posttranslationally modified to increase its apparent molecular mass to 47,000 D (Warren et al., 1979). The normal and mutant *fs(1)1163* polypeptides can be processed in vitro by dog pancreas microsomes, producing in YPI the same posttranslational increase in molecular mass and other posttranslational modifications such as glycosylation and phosphorylation. However the latter two modifications occur at

The sequence data for *Drosophila* yolk protein 1 are available from EMBL/GenBank/DBJ under accession number V00248.

a reduced rate in the *1163* (Minoo, 1982; Minoo and Postlethwait, 1985). The mutant *yp1* gene has been cloned and sequenced and has an isoleucine to asparagine substitution at position 92 in the resulting YP1 (Saunders and Bownes, 1986).

We discovered a novel phenomenon where a protein is secreted from the cells but is retained by the tissue. Normally the three polypeptides are synthesized and quickly pass into the hemolymph where they exist as multisubunit complexes (Isaac, 1982; Fournery et al., 1982). However, we find evidence that yolk proteins accumulate as electron-dense material in the subbasement membrane space, mimicking a phenomenon found in the protein uptake of the larval fat body (Butterworth and Forrest, 1984). This accumulation suggests that the mutant amino acid sequence of the yolk protein may create conformational or electrostatic modifications that prevent penetration of the basement membrane.

Materials and Methods

Genetic Techniques

The normal and mutant strains hetero-, homo-, hemizygous for the female sterile mutant *fs(1)1163* used for these experiments are described in Table 1 and in Bownes and Hodson (1980), Lindsley and Zimm (1985, 1986, 1987, 1990), Saunders and Bownes (1986), and Williams et al. (1987). The Oregon-R female has two doses of each of three, wild-type yolk protein genes, *yp1*, *yp2*, and *yp3*. Flies homozygous for the female sterile mutant gene for YP1 (*fs(1)1163*) have two doses each of normal *yp2* and *yp3* genes. In the heterozygous state *fs(1)1163* on one homologue is balanced by the *FM3* chromosome which has a complete set of normal YP genes. The *1163* allele is temperature sensitive: in the heterozygote (*1163/FM3*) the female is fertile and YP1 is secreted at 18°C, but it is not fertile, nor is YP1 secreted at 29°C. In the homozygote the females are sterile and fail to secrete YP1 at either temperature. The mutant YP1 can be made hemizygous using the deletion *df(1)C52* which includes the *yp1* and *yp2* genes. This deletion is normally maintained in a balanced lethal stock by the *FM6* chromosome. Hemizygotes of *1163* have one mutant *yp1*, one normal *yp2*, and two normal *yp3* genes. Two Oregon-R wild-type lines were transformed with the *1163* sequence incorporated into a pUChsneo plasmid, *pRS 2-p[neo;yp1¹¹⁶³]*. The sequence contains a fat body-specific and sex-specific enhancer and the coding sequence of the mutant *yp1* gene. Only one copy of *1163* has been detected in the line *trans-I*, whereas in *trans-O* more than one *1163* copy

Table 1. Subbasement Membrane Material Is Found Whenever the *1163* Gene Sequence Is Present

Genotype*	YP1 scrted [‡]	SBMM [§]	Genotype	YP1 scrted	SBMM
Wild type	Yes	No	<i>1163 trans-O</i>	No	Yes
<i>1163/FM3, 20°</i>	Yes	No	<i>1163/C52</i>	No	Yes
<i>1163/FM3, 29°</i>	No	Yes			
<i>1163/1163</i>	No	Yes	<i>C52/FM6</i>	Yes	No
<i>1163 trans-I</i>	No	Yes	<i>1163/FM6</i>	No	Yes

* Genotype: wild type Oregon-R strain; *1163/FM3*, female sterile mutant gene for YP1 (*fs(1)1163*) on the homologue balanced by the *FM3* chromosome (reared at 20 and 29°C); *1163/1163*, homozygote for the *fs(1)1163* mutation; *1163/C52*, *fs(1)1163* heterozygous for a short, X-linked deletion *df(1)C52* which includes both the *yp1* and 2 genes (Postlethwait and Jowett, 1980); *C52/FM6*, the deletion *df(1)C52* balanced by the *FM6* chromosome; *trans-I* and *trans-O*, Oregon-R wild-type strains transformed with the *1163* sequence incorporated into a pUChsneo plasmid, *pRS 2-p[neo;yp1¹¹⁶³]*. All genotypes analyzed were reared at both 20 and 29°C. Only *1163/FM3* is listed here to illustrate the strict wild-type expression at 20 and the mutant expression at 29°C; however, see text for details. See Materials and Methods and Lindsley and Zimm (1985, 1986, 1987, 1990) for further genotypic details.

[‡] YP1 scrted, yolk protein 1 secreted from the fat body into the hemolymph.
[§] SBMM, electron-dense material containing YP which accumulates in the subbasement membrane space.

is present (Saunders, R. D. C. and M. Bownes, unpublished Southern blot analysis). Females of these lines thus contain two wild-type copies of each *yp* gene and are homozygous for the additional mutant *yp* sequences. It should be noted that *p* elements insert singly, not in tandem arrays as in many other transformation systems. These mutant transformed sequences are expressed in the fat body of adult females. Further details of the production of these lines and detailed analysis of the expression of other lines can be found in Saunders (1986).

Cytological Techniques

All strains were raised at 20 and 29°C and after 3, 7, and 14 d of culture were dissected, and adult fat body was fixed in glutaraldehyde and osmium tetroxide, and prepared for electron microscopy, following methods listed in Butterworth et al. (1988). Thin sections were stained with uranyl acetate and lead citrate. In some cases morphometric analysis was performed at the light- and electron-microscopic level according to the methods in Butterworth et al. (1988). Tissues were also prepared for immunogold staining where LR White sections were treated with rabbit anti-YP and protein-A gold according to a modified method of Bendayan (1984). The tissues were fixed in a mixture of 2% glutaraldehyde (Electron Microscopy Sciences, Fort Washington, PA) and 0.5% paraformaldehyde (Polysciences, Warrington, PA), dehydrated in alcohol, stained en bloc with uranyl acetate and embedded in LR White (Polysciences). Thin sections were incubated in dilute sections of anti-YP and subsequently treated with protein-A gold (Janssen, Life Sciences Products, Piscataway, NJ). Controls were of three types: (a) sections of fat body received similar treatment except the anti-YP step was omitted; (b) tissues known to synthesize and contain yolk protein (mature oocytes) were stained with both steps; and (c) tissues that neither synthesize nor contain yolk (muscle) also received both steps. In controls (a) and (c) very low amounts of gold deposits were observed as expected from random, nonspecific staining. In control (b) very high levels of gold grains were observed in yolk granules; up to 70 times higher than equally-sized areas of surrounding ooplasm (see Fig. 7 C).

Preparation of Antiserum

Antibody specific to yolk proteins of *Drosophila* was prepared by alternately injecting rabbits with YP purified from eggs by ammonium sulfate precipitation and then with YP bands eluted from SDS-PAGE gels. This alternate injection was repeated for two cycles effectively eliminating many cross-reacting, nonspecific proteins as evidenced by Western blot analysis (see Fig. 1). Freund's complete adjuvant was included in the first inoculum, and for all subsequent injections Freund's incomplete adjuvant was used.

Results

Wild Type

The fat body cells in normal female flies raised for 3, 7, and 14 d at either 20 or 29°C contain massive reserve deposits

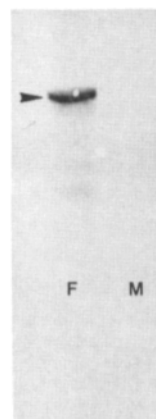


Figure 1. The purity of the YP (arrowhead) antibody is shown by Western blotting of adult female (F) and male (M) extracts.

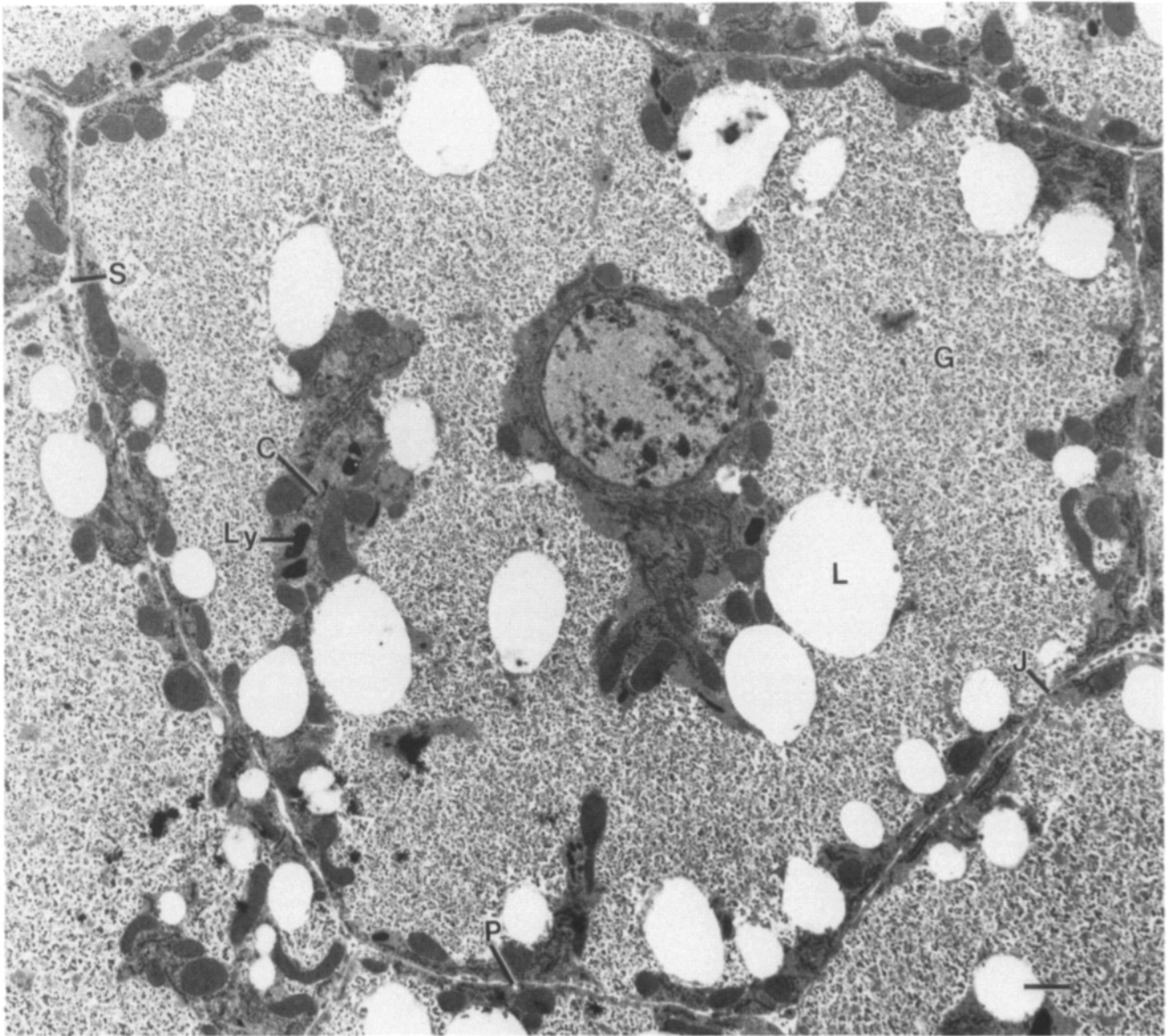


Figure 2. Electron micrograph of a typical fat body cell from a 14-d, wild-type female reared at 29°C showing major components present in the mature cell: glycogen (G), Golgi complexes (C), lipid (L), lysosomes (Ly), gap junctions (J), empty coated pits (P), and subbasement membrane space (S). Golgi complexes were identified as areas of cytoplasm, devoid of RER and reserve deposits, which possess features such as *trans*- or *cis*-vesicles, lamellar stacks of smooth membranes some of which contain electron-dense material in the lumens. The only stored materials are glycogen (massive, granular area) and lipid droplets (empty circular spaces whose contents were extracted by cytological preparation methods). With the exception of a few electron-dense bodies, most probably lysosomes, there are no storage vesicles for yolk protein, confirming the earlier results of SDS-PAGE analysis (Bownes and Hodson, 1980) and the light microscopic observations of Butterworth and Bodenstern (1968). No differences between animals reared at either temperature were observed. Bar, 1 μ m.

of lipid and glycogen: lipid accounting for ~25% of the cell and glycogen, 60%. The lipid is in the form of droplets ranging in size from 0.5–4 μ m, but occasionally up to 10 μ m in diameter. The nonreserve cytoplasm is found along the plasma membrane, the nuclear membrane, and randomly extends as strands into the masses of glycogen (see Fig. 2). The cells in cross section are often polygonal in shape connected to adjacent cells by numerous gap junctions. Between junctions the plasma membranes are separated, forming irregularly shaped, intercellular spaces. The plasma membrane that is exposed to the hemolymph is coated with a basement membrane. In all cases there is a space between the plasma membrane and the basement membrane called the sub-

basement membrane space which is continuous with the intercellular spaces. The basement membrane makes the cell surface–hemolymph interface appear smooth, but the plasma membrane with occasional coated pits is convoluted, thus significantly increasing the cell surface area. The nonreserve cytoplasm contains numerous mitochondria, RER, free ribosomes, Golgi complexes, endocytic vesicles, lysosomes, and occasional microtubules. The lumen of the RER often contains material of medium electron density sometimes causing the membranes to become relatively large spheres or tubules in cross section. Golgi complexes often have several Golgi stacks where some cysternae are filled with electron-dense material, putative transport vesicles, and

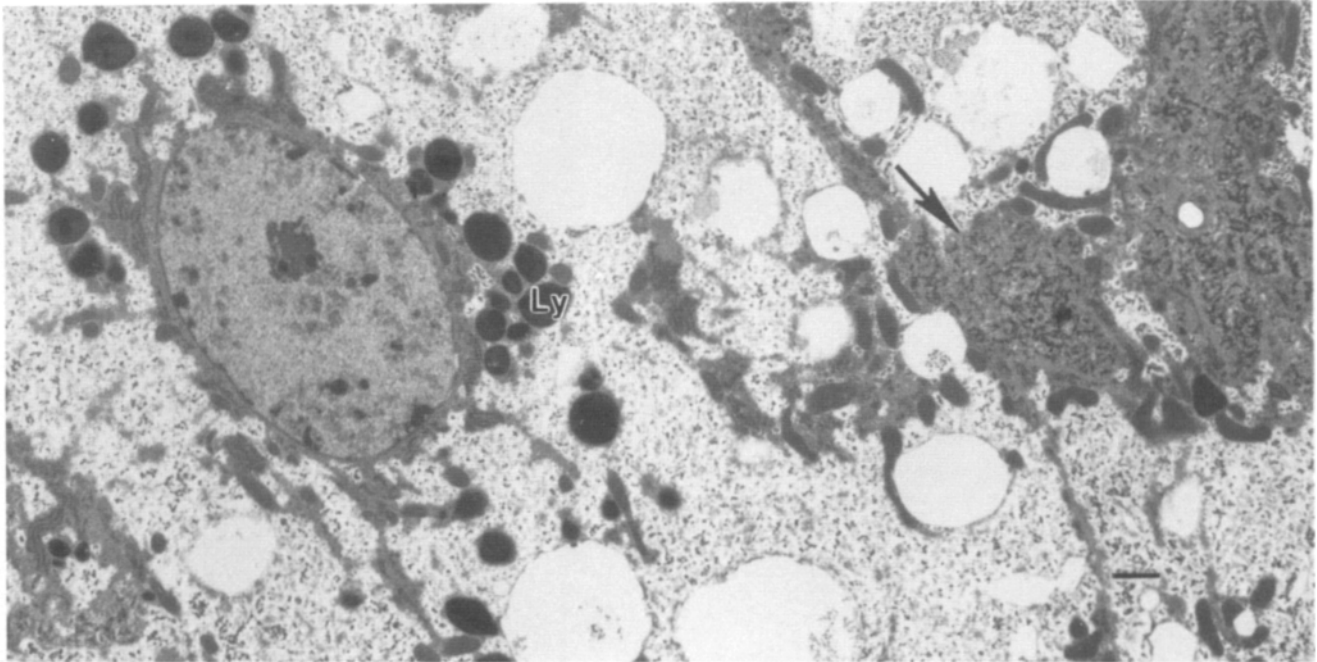


Figure 3. Electron micrograph of typical fat body cells from a 14-d, *fs(l)1163* mutant, female homozygote at 29°C showing in addition to the structures indicated in Fig. 2 (above) electron-dense foci of SBMM (arrow). These SBMM foci are typically located near the plasma membrane, but can become massive, extending in plasma membrane-bounded tubules, displacing the cytoplasm, and in some cases filling a significant proportion of the cell (see Figs. 5 and 6, below). This SBMM accumulation is observed in all the genotypes studied, containing the *fs(l)1163* mutation suggesting that it is yolk (see Table I). *Ly*, lysosomes. Bar, 1 μ m.

electron-dense bodies (see Fig. 9 A) in a cluster surrounded by RER. Lysosome-like bodies ranging in size up to 1 μ m are often not closely associated with the Golgi complexes and comprise \sim 2% per unit area of normal tissue. This ultrastructural profile of wild type is consistent among all three ages studied and both temperatures evaluated.

Protein storage granules are not observed, which is consistent with the earlier, biochemical (Bownes and Hodson, 1980) and light microscopic studies (Butterworth and Bodenstern, 1968), prompting the conclusion that YP is secreted directly into the hemolymph. As we will show below YP is secreted into the subbasement membrane space where

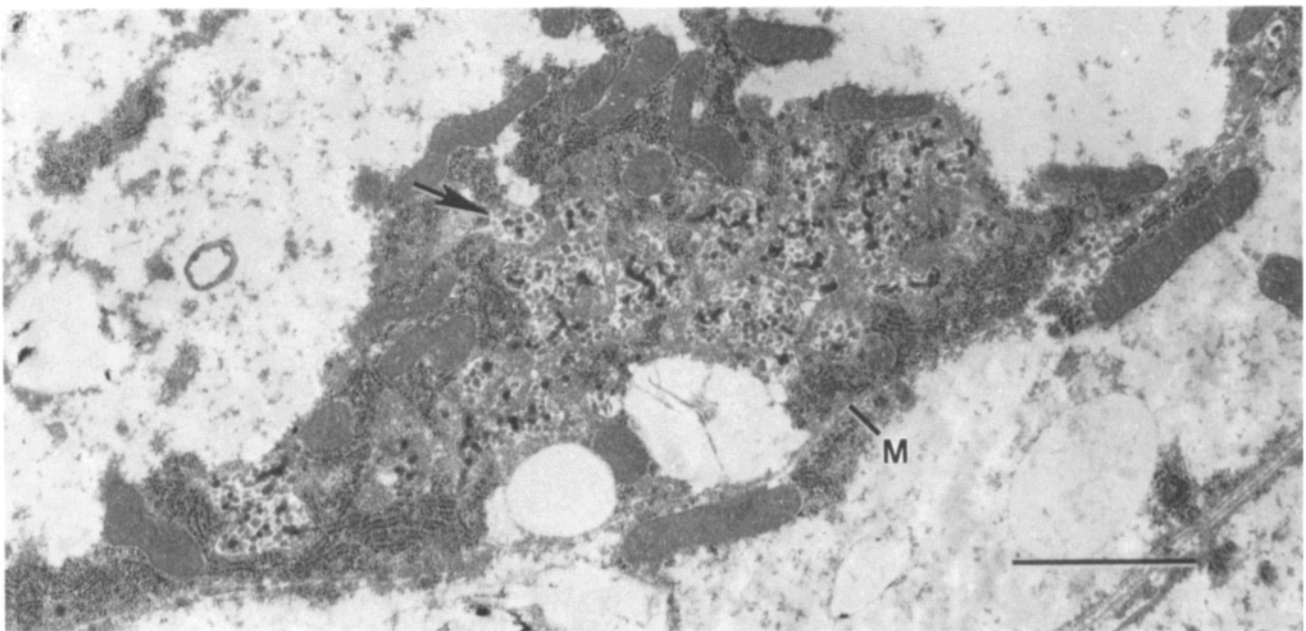


Figure 4. An enlargement of one of the SBMM foci in *fs(l)1163/FM3* tissue from a 14-d, heterozygous female raised at 29°C. Note that this mass is composed of subfoci (arrow) that are interpreted as tubular invaginations of the plasma membrane (*M*), and that the SBMM consists of two types of electron density: low and high. Bar, 1 μ m.

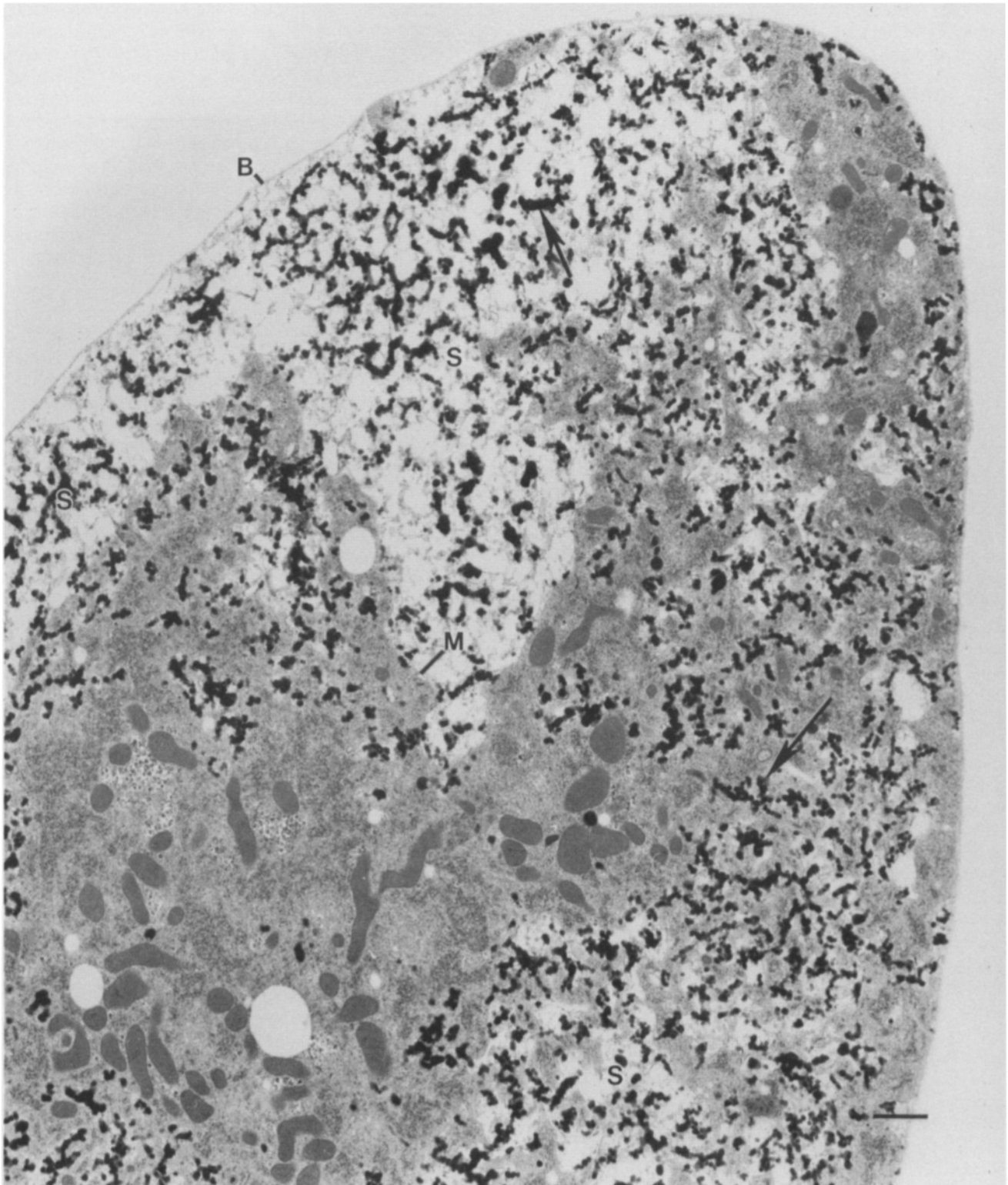


Figure 5. Electron micrograph of a fat body cell from a *fs(1)1163* female (14 d, homozygote at 29°C) displaying massive accumulation of SBMM (arrows) and subbasement membrane space (S) comprising nearly 50% of the cell. This extreme form suggests SBMM, unable to penetrate the basement membrane (B), displaces cytoplasm, creating extensive increases in the amount of the plasma membrane (M). Note that in these instances relatively little reserve deposits are seen. Although the cell represents an extreme of SBMM accumulation, many cells have no SBMM. Bar, 1 μ m.

it diffuses through the basement membrane and into the hemolymph. Because the issue is found in multicellular clumps along the inner periphery of the exoskeleton, it is reasonable to conclude that the yolk protein that is secreted by interior cells will pass along intercellular channels that comprise the subbasement membrane space.

1163/FM3

Animals that are heterozygous for *1163* have temperature-dependent expression of the phenotype, where the females are fertile at 20°C. At 20°C the cell size and amounts of reserves at both the light and electron microscope levels are morphologically similar to that of wild-type females. In earlier studies Bownes and Hodson (1980) using SDS-PAGE gels indicated that the cells contain the same low levels of yolk proteins as normal cells. However, at 29°C there is a significant change in expression of the *1163* trait. Their electrophoretic gels showed that the mutant YP1 failed to be secreted and instead was accumulated in the fat body. The secretion of the normal YP2 and YP3 proteins and also YP1 from the single wild-type gene was hindered, and all three YPs accumulated in this tissue. The cells are 44% smaller than those of animals raised at 20°C due mainly to reserve loss. At the fine structure level, unusual electron-dense material is found in the subbasement membrane space of many cells (see Figs. 3 and 4). This subbasement membrane material (SBMM)¹ is often located near the plasma membrane in foci or smaller subfoci. In sections which show no SBMM the blocks were resectioned and examined. Here SBMM was found in the newer sections indicating that the material is either produced in a few select cells or it is produced in many cells but accumulates only in certain cells. Although some cells have no SBMM, some cells have massive amounts which comprise nearly 50% of the cell volume (see Fig. 5). In other cases the SBMM appears in tubular inlets throughout the cell (see Fig. 6 A).

This SBMM is of two types: low and high electron-density material (see Figs. 4 and 6, A and B). The low density SBMM at higher magnification is occasionally fibrous with a crystalline, lattice-shaped morphology and the high density material is amorphous (see Fig. 6 B). Often the high density SBMM is found in clathrin-coated pits and endocytic vesicles (Fig. 6 B) suggesting there may be receptors for this material. However, fluid-phase uptake may also occur. Since the low density SBMM is not seen in these pits and endocytic vesicles, we propose that either the low density SBMM precedes the appearance of the high density material or that there are no receptors for the low density material. Immunogold analysis using antibody to YP indicates that SBMM contains yolk, and that it is taken up by coated pits (Fig. 7, A and B). The high specificity of the antiserum is indicated in Figs. 1 and 7 C. As indicated in Table I the presence of SBMM in the fat body, and the reduced ability of these cells to secrete YP1 is correlated with the presence of the *1163* mutation.

That SBMM is being internalized by cells is further suggested by examples of lysosomes that contain small, electron-dense masses morphologically similar to SBMM

1. *Abbreviation used in this paper:* SBMM, subbasement membrane material.

(see Fig. 8). Furthermore there is a positive correlation of the amount of SBMM and number of 0.5–1 μm diameter lysosomes ($r = 0.58$, $n = 22$, significant at 1%) further suggesting the destruction of SBMM by lysosomes. This correlation includes 22 paired sets of averaged amounts of lysosomes and SBMM detected in electron micrographs from most of the *1163*-containing constructs listed in Table 1 at the times and temperatures where morphometric data could be obtained. Golgi complexes in the mutant animals have similar morphologies to those of wild type, and their position in the cell is evenly distributed (see Fig. 9) as in wild type suggesting no visible differences in the secretory apparatus.

1163/1163

Flies homozygous for *fs(1)1163* reared at either 20 and 29°C have a phenotype similar to that of *fs(1)1163* heterozygotes reared at 29°C (see Table 1). All three classes display the smaller cell size, the presence of SBMM, appearance of YPs in the fat body gels, and female sterility. Furthermore the amount of SBMM in the older animals raised at 29°C is the largest of all the *1163* constructs listed in Table 1 (17 and 37% per unit area of the tissue analyzed of 7- and 14-d animals, respectively) suggesting that the reabsorption of SBMM is inefficient.

Thus a specific cellular morphology in the fat body has been correlated with the presence of *ypl* mutant gene and is consistent with the molecular and biological data on sterility. To observe the effects of the mutant *ypl* gene product in the absence of a wild-type *ypl* gene, hemizygous mutant flies were analyzed.

1163/C52

The females of *1163* strains, that were constructed with the short deletion for *ypl* and *yp2* on one homologue, are hemizygous for the mutant *ypl* and normal *yp2* genes; but both copies of *yp3* are wild type. Here the fat body is very abnormal, displaying only minute amounts of reserves. Nevertheless the cells are active, containing RER, normal-looking Golgi complexes and large numbers of lysosomes. In addition SBMM is present in large amounts in the older animals reared at the higher temperature (12 and 24% per unit area of the tissue of 7- and 14-day animals, respectively, at 29°C). Thus the presence of SBMM seems to be directly related to the *1163* gene. However, it is possible that there are other mutations in the stock contributing to other cellular abnormalities of this phenotype. To test whether the results were the direct result of the mutant *ypl* gene, it was transformed into a wild-type background and the morphology of the cells was analyzed.

trans-I and trans-O

Oregon-R flies were transformed with the plasmid *pRS 2-p[neo;yp1¹¹⁶³]*, and two lines were studied: *trans-1* which has been shown to contain one copy of the *1163* gene, and *trans-O* which has more than one copy (Saunders, R. D. C., and M. Bownes, unpublished data). The transformed sequence contains a fat body-specific and a sex-specific enhancer, and the mutant YP1 protein is expressed in the fat body of the transformed females, as evidenced by a similar retention of the YPs in the fat bodies at 29°C on SDS-PAGE gels. Qualitative observations at the light and electron micro-

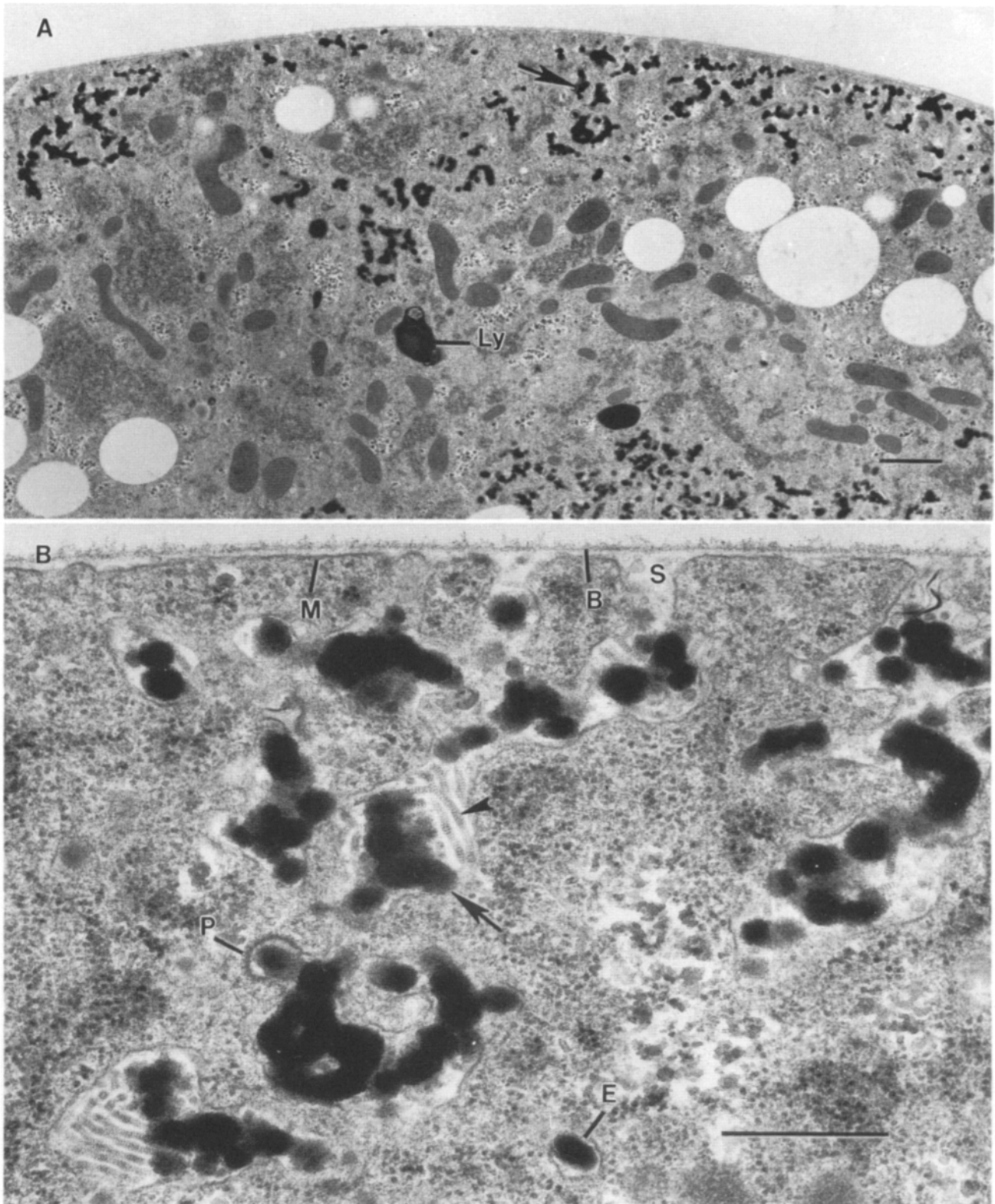


Figure 6. Electron micrographs illustrate a proposed mechanism of accumulation of SBMM in the mutant tissue (14 d, homozygote at 29°C). (A) At low magnification SBMM (arrow) is found at the cell/hemolymph periphery, but also internally. (B) A higher magnification detail of A above clearly shows SBMM in the subbasement membrane space (S), that the low electron-density form has a lattice structure (arrowhead) and the high density form is amorphous (arrow). Note that the high density SBMM is being engulfed by a coated pit (P). This figure suggests that the mutant protein is secreted into the subbasement membrane space, but because of the amino acid substitution in YP1, the oligomers containing YP1 cannot penetrate the basement membrane (B). Perhaps, as the concentration of oligomer increases, the crystalline, lattice-like fibers form which collapse into amorphous masses, which in turn become resorbed by coated pits, and the resulting endosomes (E) fuse with lysosomes (Ly). The basement membrane morphology of wild type is no different from that of the mutant. M, plasma membrane. Bars: (A) 1 μm ; (B) 0.5 μm .

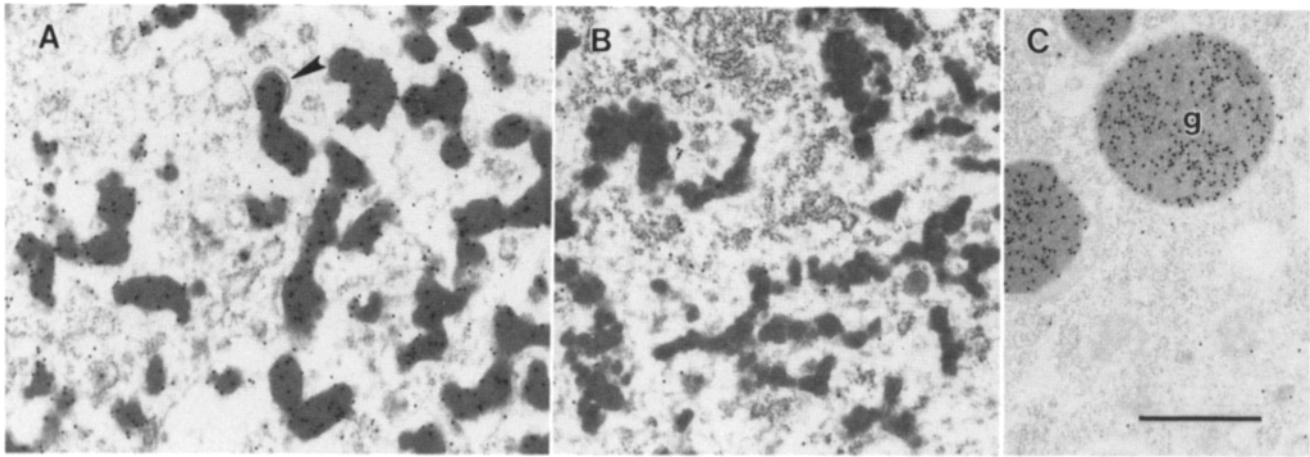


Figure 7. Electron micrographs of mutant tissue (7 d, homozygotes at 29°C) stained by the immunogold procedure. (A) Experimental section of fat body indicates abundant levels of 15-nm gold grains over SBMM. Note labeling of SBMM in a coated pit (*arrowhead*). (B) Control section of fat body where anti-YP is omitted demonstrates SBMM has low levels of gold particles relative to the background cytoplasm. (C) Positive control of an oocyte showing heavily labeled yolk granules (g) and low levels of labeling in the surrounding ooplasm. Bar, 0.5 μ m.

scopic levels indicate that the fat body appears normal in morphology, but does contain SBMM in low to moderate amounts. In the *trans-I* line SBMM is present in low amounts and only in 3-d animals at both temperatures. In *trans-O* animals there are significant amounts of SBMM at the higher temperature in the 7- and 14-d animals.

Discussion

Previous studies (Bownes and Hodson, 1980) have shown that the *fs(1)1163* mutation in the *ypl* gene suppresses secretion of YP1 and reduces secretion of yolk peptides 2 and 3 from the fat body into the hemolymph. The current studies

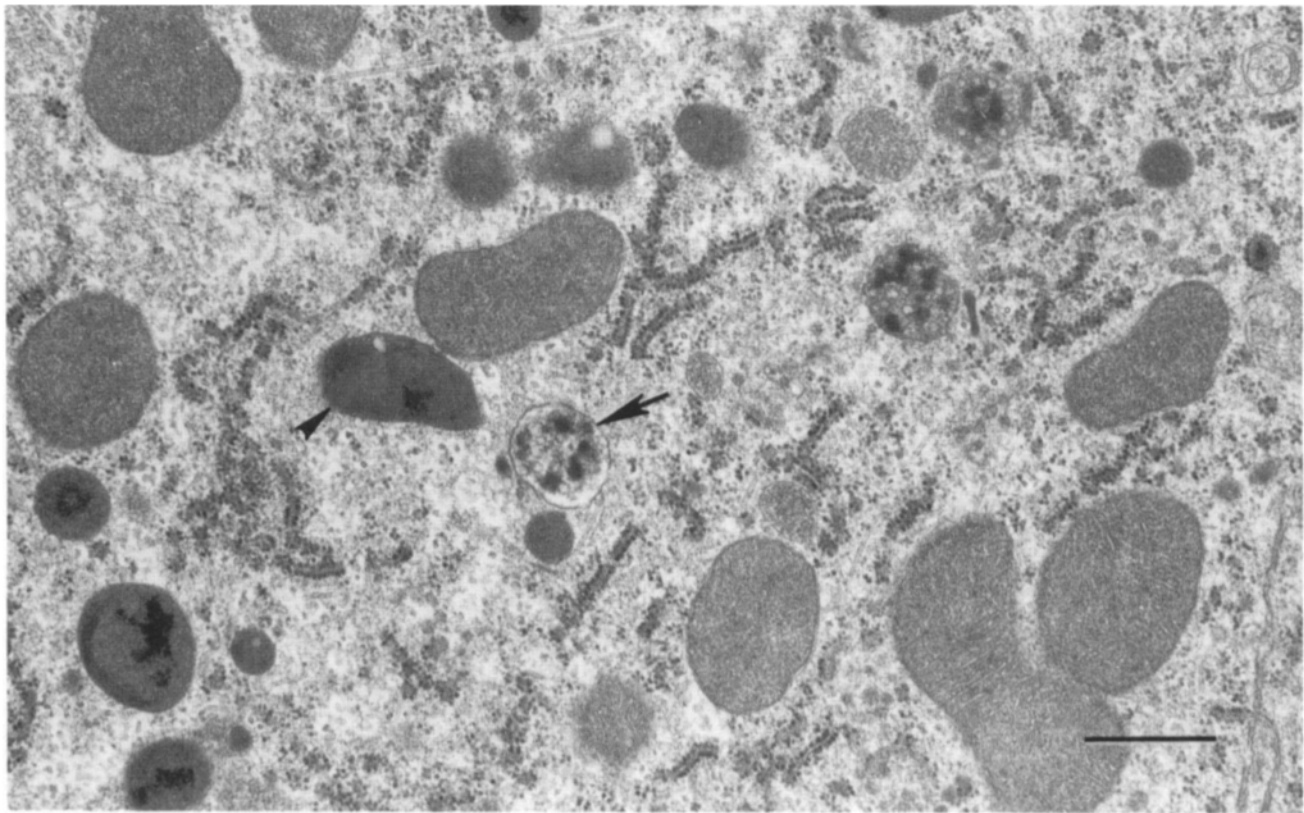


Figure 8. Electron micrograph of lysosomes (*arrow*) in the mutant fat tissue (7 d, heterozygote at 29°C) that contain dense material similar in morphology to the SBMM in endosomes not seen in normal tissues. Note other lysosomes perhaps at a later stage of maturation (*arrowhead*). Morphometric analysis indicates that in the mutant, the amount of SBMM per cell correlates positively with the number of lysosomes, suggesting that these cells are engaged in the removal of SBMM. Indeed, some of the lysosomes (*arrow*) appear similar to multivesicular bodies reported by Locke and Collins (1968), which they showed to contain endocytosed material. Bar, 0.5 μ m.

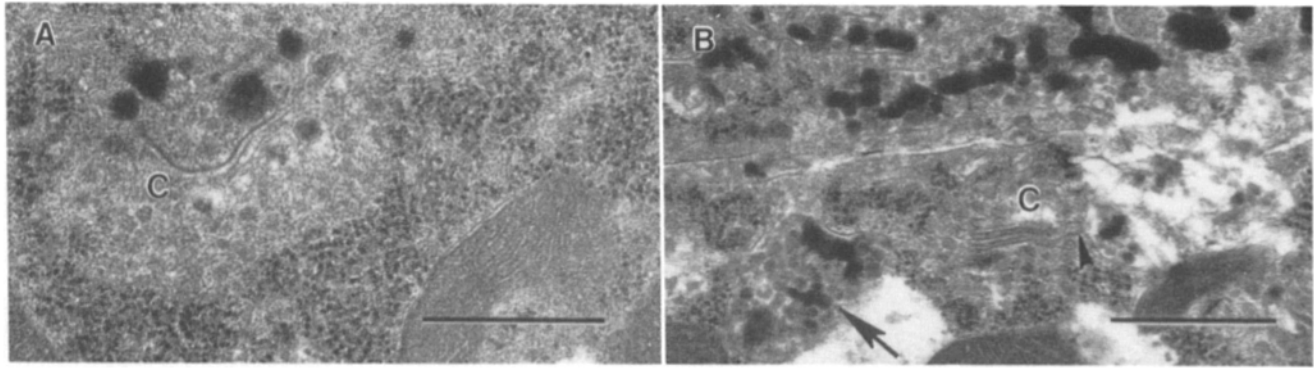


Figure 9. Electron micrographs of examples of Golgi complexes found in mutant tissues. In all genotypes, ages, and temperatures studied Golgi complexes have similar morphologies and are found relatively evenly distributed throughout the nonreserve cytoplasm. (A) Golgi complex (C) found in the internal part of the cell (7 d, homozygote at 20°C). (B) Golgi complex located adjacent to the plasma membrane (3 d, heterozygote at 29°C). Note what appears to be a *trans*-vesicle (arrowhead) fusing with the plasma membrane. SBMM (arrow); Bars, 0.5 μ m.

indicate that YP secretion from the fat body cells is normal in the *fs(1)1163* mutation. However the amino acid substitution in YP1 causes the novel accumulation of electron-dense SBMM in the subbasement membrane space, thus retaining the protein in the fat body tissue. It is likely that SBMM contains yolk protein because (a) SBMM is never found in wild-type cells and (b) this material is found in all cases where the *1163* gene is present (except at 20°C in the heterozygote), and (c) that it is positive for yolk in immunogold analysis. Finally we have shown clearly that this phenotype is a direct result of the mutant *1163 ypl* gene, since wild-type flies carrying the mutant *ypl* gene introduced by transformation with P-element vectors also show this abnormal phenotype.

Assuming that SBMM is a condensed form of a YP oligomer, the ultrastructural evidence prompts us to speculate that all three yolk proteins are secreted normally, but because of a charge difference or a conformational change in YP1, YP oligomers containing mutant YP1 cannot pass through the basement membrane. It is possible that other proteins secreted by the fat body may become associated with these aggregates also. Conceivably the oligomer continues to build in concentration until crystallization begins (the low density lattice-like fibers in Fig. 6 B), which in turn collapses into the electron-dense, amorphous masses. Alternatively the SBMM aggregate forms immediately after secretion, and the aggregate is trapped by the basement membrane. Finally, evidence is presented that suggest that SBMM is endocytosed and degraded by lysosomes. Immunogold studies coupled with morphometric analysis are underway to evaluate the yolk contents of various morphological classes of lysosomes, the vesicles of the Golgi complexes, and the high and low density SBMM to gain better insight into the secretory traffic of the mutant yolk.

Endocytosis of SBMM through coated pits could be receptor mediated. Since YP receptors for YP endocytosis exist in the oocytes of insects (Mahowald, 1972; Raikhel, 1984a, b, 1987; Rorhkasten and Ferenz, 1986; Konig and Lanzrein, 1985; and Osir and Law, 1986), such a receptor system may be present in the coated pits that endocytose SBMM in the *1163* fat body. However, the function of specific YP receptors in the normal fat body is unclear, thus it seems more likely that SBMM endocytosis may be a general process for protein

aggregates that accumulate in the subbasement membrane space (see discussion of the larval fat body below).

Although animals with the *1163* gene possess SBMM, only a low proportion of cells actually contain the material. One possibility is that only a few cells at any given time are in the process of synthesizing yolk protein, though this is unlikely, since studies of YP transcripts show they are present in all fat body cells (Gillman, C., and M. Bownes, unpublished data). Another possibility is that mutant yolk protein may not be secreted but shunted through the lysosomal pathway as explained by the larger number of lysosomes present. Mechanisms have been described which show that proteins with abnormal sequences are destroyed in the cytosol (Dice, 1987; Goldberg and Goff, 1986). Thus SBMM may be the result of an escape of a small amount of mutant YP1 from an overloaded degradative system. A further possibility is that all cells synthesize and secrete yolk protein into the extensive subbasement membrane space. However, only where the YP1 concentration becomes sufficiently high, does it precipitate or crystallize resulting in a relatively low number of foci of SBMM.

Normally secretory vesicles fusing to the plasma membrane could cause a net increase in the amount of plasma membrane if it were not for the phenomenon of membrane recycling (Herzog and Farquhar, 1977; Snider and Rogers, 1986; Rothman and Schmidt, 1986; Duncan and Kornfeld, 1988). A characteristic of SBMM-containing cells is the extensive invagination of the plasma membrane, forming tubules of SBMM reaching far into the cell suggesting the plasma membrane is not recycled. Furthermore animals producing SBMM have significantly smaller fat cells, the result of reserve loss. Perhaps this loss is associated with the failure of membrane recycling. There may be some recycling through the endocytosis of SBMM, but this process is inefficient since amounts of SBMM tend to accumulate with age.

Formation of SBMM in the *1163* adult fat body has similar features to protein granule formation in the normal, larval fat body (Butterworth and Forrest, 1984). The larval cells 24 h before metamorphosis sequester blood proteins that are condensed into granules. However, ultrastructural and morphometric analysis of this process shows that granule forma-

tion actually begins in the subbasement membrane space (Butterworth and Forrest, 1984). Masses of low electron density appear first, which then condense into high electron-density granules, which then are endocytosed by coated pits. Condensation of protein solutions in the subbasement membrane space in two separate tissues, the larval and adult fat body, suggests that common enzymatic machinery exists in both tissues. It should be noted that in *Drosophila* the larval fat body histolyses at eclosion, and new cells differentiate into the adult fat body during the first day of adult life. Perhaps only when confronted with mutant protein aggregates, does the adult tissue machinery become activated. Possibly this mechanism occurs in the mammalian kidney. When solutions of heparin and protamine were injected separately into rats (Seiler et al., 1975; Schwartz et al., 1981; Sonnenburg-Hatzopoulos et al., 1984), the two proteins formed an aggregate which condensed within the glomerular basement membrane. Growing in size the aggregates passed through the membrane and into the urinary sinus where they formed granules that were endocytosed.

It is a widely held belief that temperature-sensitive mutations have their effect on protein activity by affecting the protein conformation. The temperature-dependent effect on secretion of the *1163* YP suggests that the cause may be related to conformational abnormalities thus preventing the normal passage of YP1 from the cell to the hemolymph. Furthermore female sterility is not a direct result of the *1163* YP1 being expressed in the fat body, since some of the transformed lines carrying this mutant gene are fertile (Saunders, R. D. C., and M. Bownes, unpublished data). It seems likely that the female sterility is caused by the abnormal secretory process in the follicle cells initiated by the putative folding defect of YP1. This conclusion is consistent with observations of Giorgi and Postlethwait (1985). They found that YP1 secretions in the follicle cells of the *1163* mutants interfered with vitelline membrane formation, leading to decreased oocyte integrity, a possible cause of sterility.

An intriguing relationship may exist between deoxyhemoglobin S of sickle cell anemia and the *1163* mutant YP1. It is well known that hemoglobin S differs from hemoglobin A by a single substitution of valine (a nonpolar amino acid) for glutamic acid (a polar molecule), that valine, located on the outer surface of the molecule creates a "sticky" patch, and at low oxygen tension a hydrophobic site is created between the E and F helices in deoxyhemoglobin S which is complementary to the sticky patch. Thus deoxyhemoglobin S molecules bind together to form fibers that create the sickling phenomenon in the red blood cells (Finch et al., 1973; Edelstein et al., 1973; Dykes et al., 1978). These fibers are of two types: the more prevalent 20-nm size with diagonal striations and the 17-nm size with perpendicular striations. The conformational event of sticky and complementary binding sites may occur also in *1163* YP1 causing SBMM to form. In the case of *1163* the amino acid substitution of polar asparagine is for nonpolar isoleucine. Further the SBMM having low electron density (Fig. 6 B) has a fiber structure similar to that of the 17-nm deoxyhemoglobin S fibers. The SBMM fibers are 17 nm thick and have perpendicular striations. Whether a sticky and complementary site occur in *1163* YP1 will have to await x-ray crystallography.

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