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Germ Line and Soma Cooperate during Oogenesis to Establish the Dorsoventral Pattern of Egg Shell and Embryo in Drosophila melanogaster

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Summary

Mutations in *gurken* and *torpedo* cause a ventralization in the follicle cell epithelium during Drosophila oogenesis and in the pattern of the embryo that develops in the resultant egg. Both genes lie midway in an epistatic series between *fs(1)K10* and *dorsal*; the mutations block the dorsalization normally observed in *K10* eggs but have no effect on the phenotype of embryos derived from *dorsal* mothers. Analysis of germline mosaics demonstrates that both ovarian and embryonic phenotypes will be produced when either the *gurken*$^+$ gene is removed from the germ line or *torpedo*$^+$ is removed from the soma. This shows that the dorsoventral pattern of the Drosophila egg chamber depends on the transfer of spatial information from the germ line to the somatic follicle cells, and from somatic cells to the oocyte.

Introduction

In the eggs of many species, visible asymmetries can be observed at the end of oogenesis. Often these asymmetries correlate well with the primary axes of the developing embryo, suggesting that the mechanisms used during oogenesis to build an asymmetric egg are also responsible for the establishment of an asymmetric embryo. In most cases, however, it is not known how the visible asymmetries of the egg architecture relate to the primary body plan of the embryo. In Drosophila these asymmetries are particularly pronounced in the pattern of the outer layer of the egg shell, the chorion, but they are also visible in the shape of the egg and the location of the micropyle. These asymmetries arise during oogenesis and reflect the spatial arrangement of the oocyte and its helper cells within the developing egg chamber (for detailed description see King, 1970; Mahowald and Kambysellis, 1980; Margaritis et al., 1980; Margaritis, 1985).

Maternal-effect mutations have been described in Drosophila that alter the anterior-posterior or the dorsoventral axis of the embryo without changing the shape of the egg or the pattern of the chorion. (Bull, 1966; Gans et al., 1975; Nüsslein-Volhard, 1977; Nüsslein-Volhard et al., 1980; Anderson and Nüsslein-Volhard, 1984; Boswell and Mahowald, 1985; Degelmann et al., 1986; Frohnhöfer and Nüsslein-Volhard, 1986; Lehmann and Nüsslein-Volhard, 1986; Mohler and Wieschaus, 1986; Schüpbach and Wieschaus, 1986a). At some point in development, the maternal information that determines the embryonic body pattern is therefore independent of the maternal information that determines egg shape or the pattern of the chorion. A few mutations in Drosophila are known, however, that cause parallel changes in the pattern of the egg chamber and the chorion as well as in the pattern of the embryo—viz. *fs(1)K10* (Wieschaus et al., 1978; Wieschaus, 1979, 1980) and *dicephalic* (Schardin, 1982; Frey and Gutzeit, 1986). The corresponding wild-type genes seem to participate in processes that link the asymmetries of egg chamber and embryo.

This present report describes two new genes—*gurken* (*grk*) and *torpedo* (*top*)—where mutations will also interfere with processes that link egg morphology with the pattern of the embryo. Mutations in both genes lead to a ventralization of the egg and the embryo. In addition, the analysis of germ-line mosaics demonstrates that in Drosophila, gene activity of the maternal somatic cells as well as the germ line is required for the formation of a normal embryo.

Results

Females homozygous for *grk* or *top* produce eggs with very similar alterations in the pattern of the egg shell and the pattern of the embryo. These alterations can be described most simply as ventral shifts of cellular fates, i.e., as "ventralization" of the pattern, in that ventral regions of the chorion and the embryo become expanded at the expense of dorsal regions. The mutations are completely recessive and fully penetrant in homozygous females. Six alleles of *grk* (2-30) and one allele of *top* (2-100) were isolated in mutagenesis experiments in which the mean allele frequency per locus was 2.5 (Schüpbach and Wieschaus, unpublished). Since no chromosomal deficiencies are presently available for either locus, it is not possible to test whether any of the recovered alleles represent the amorphic condition. For *grk*, the six alleles can be ordered according to the strength of the mutant phenotype, suggesting that the three strong *grk* alleles may be close to the amorphic state of the gene. The single allele of *top* produces the same range of phenotypes as the weakest allele of *grk*. Since only one *top* allele is available, it is possible that the pattern alterations caused by the mutation are not due to absence of *top* gene product in mutant females. Regardless of whether the *top*^{1} allele causes gain or loss of torpedo gene function, the *top*^{1} mutation interferes in some way with normal processes that are required for the formation of egg and embryo.

In the embryo the pattern alterations produced by *grk* and *top* are readily visible at the beginning stages of gastrulation, when a much-enlarged mass of mesodermal cells invaginates on the ventral side of the embryo (Figure 1). Instead of showing the single ventral furrow of the wild-type embryo, the enlarged mesoderm of the mutants is often organized in two ventral furrows (Figure 1b). At later stages this organization is lost and a mass of mesodermal cells fills the ventral half of the embryo. The ectodermal primordium is shifted dorsally in the mutants, and the dorsal-most cells, which would form the amnion-serosa in

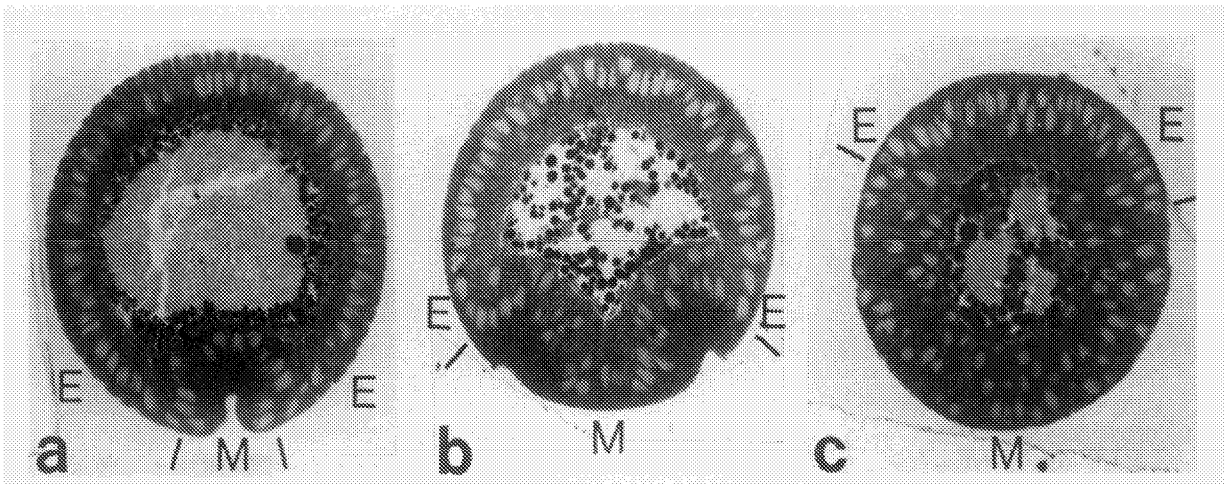

Figure 1. Gastrulating Embryos in Cross Section

(a) In a wild-type embryo the ventral-most 16%–20% of the cells in the blastoderm circumference invaginate along the ventral furrow and form mesoderm (M); the rest of the cells are ectodermal (E). In (b), *top*, 40% of the cells in the circumference invaginate and form mesoderm. These cells are organized into two ventral furrows. This organization is lost at later stages, as seen in (c), a somewhat older embryo derived from *grk*. The dividing mesoderm cells now occupy 60%–70% of the circumference, and ectodermal cells are only found along the dorsal side of the embryo. At later stages these dorsal ectodermal cells will form cuticle carrying ventral setae, pattern elements that are characteristic of ventrolateral cells in the wild-type embryo (Lohs-Schardin et al., 1979). Magnification 250×.

a normal embryo, behave like lateral ectodermal cells (Figure 2). At differentiation, the embryo forms only a stripe of cuticle running along its dorsal midline, whereas its lateral and ventral sides contain only mesoderm, which does not give rise to cuticle. Although the embryos are never normal enough to hatch, the degree of ventralization is somewhat variable and can be quantitated using the pattern seen in the differentiated cuticle (Figure 3).

The effects of the two mutations on the chorion can also be explained primarily as a ventralization. In the weakest phenotypes the two respiratory appendages normally found in dorsolateral positions on wild-type eggs are fused into a single structure inserted directly on the dorsal midline (Figure 3b). In the intermediate phenotypes this appendage is shortened and the adjacent operculum is also reduced in size (Figure 3c). In extreme mutant eggs, dorsal appendage material is totally absent and only the ventral-most region of the operculum remains (i.e., the ventral collar of the wild-type operculum, which in the mutants closely encircles the micropyle [Figure 3d, Figure 4c]). The reduction in dorsal pattern elements is correlated with an increase in the number of follicle cells that will give rise to the main body of the chorion. Cell counts made on the main body of extreme *grk* eggs indicate that about 200 additional cells participate in the formation of the main body of the *grk* chorion (see Figure 4). *grk* and *top* eggs are in fact somewhat longer than wild-type eggs. The extent of the presumed ventralization that is seen in the chorion correlates with the degree of embryonic ventralization (Figure 3).

In addition to the ventralization of the chorion, both *grk* and *top* affect the final anterior-posterior chorion pattern. In most mutant eggs the dorsal appendage appears shifted to a more posterior position as measured in percentage of egg length (Figure 3). In addition, in many eggs produced by females mutant for *grk*, a second micropyle and a small structure resembling a patch of operculum is visible at the posterior end (in 30% of the eggs from grk^{WG}, in 70% of the eggs from grk^{HK}; Figure 2e).

Requirements for Wild-Type Gene Expression Tested in Germ-Line Mosaics

Mutations in *grk* and *top* interfere with dorsoventral patterning processes that occur during oogenesis. To distinguish the cell type in which these processes take place, mosaic females were analyzed. In Drosophila it is possible to construct mosaic females in which the germ cell and sister nurse cells are of different genotype than the surrounding somatic cells, including the somatically derived follicle cells (Van Deusen, 1976; Wieschaus and Szabad, 1979; Wieschaus, 1980). Such mosaics distinguish whether a gene is required in the germ-line derivatives or in the somatic cells of the female.

Germ-line mosaics for both *grk* and *top* were constructed, and the mosaic females were tested for production of mutant eggs and embryos. Surprisingly, the two genes behaved differently from each other (Table 1). *grk* gene activity is required in the germ line. Mutant eggs and embryos were produced only in those mosaic females whose germ-line derivatives were homozygous mutant (Table 1, series 1 and 2). They were not produced when the somatic tissues of the females were mutant but the germ-line derivatives were wild type (Table 1, series 3). *grk* therefore appears to be required for processes occurring "inside" the nurse cell–oocyte complex. These processes are of relevance for chorion patterning as well as embryonic patterning.

The *top* mutation, on the other hand, interferes with processes occurring in somatic cells. The mutant phenotype was not produced in eggs or embryos when the germ-line derivatives were homozygous mutant, but both phenotypes were evident in those mosaics possessing wild-type germ cells surrounded by mutant somatic cells. Given that this was an unexpected result, several inde-

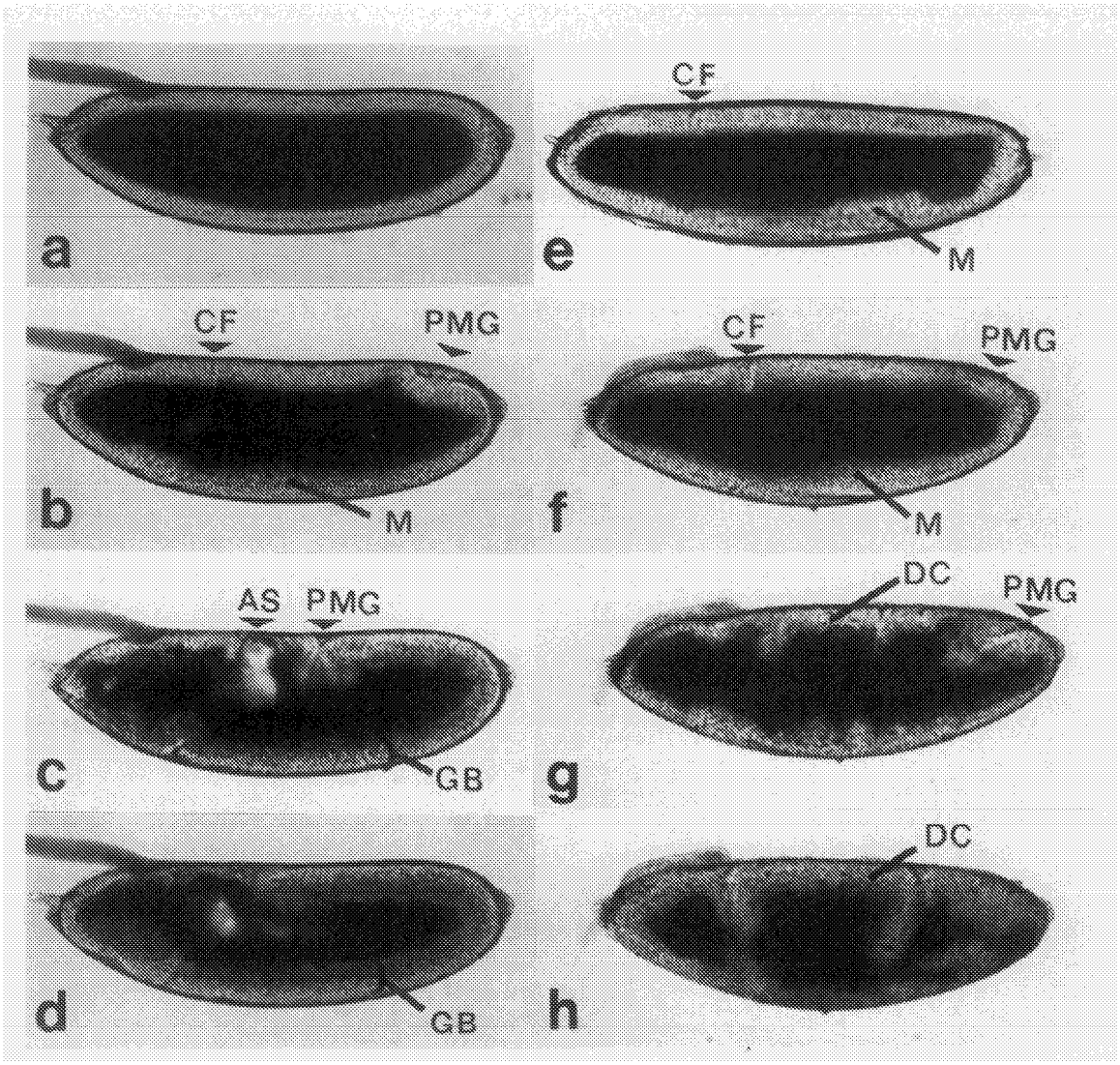

Figure 2. Living Embryos Photographed under Oil during Gastrulation

(a–d) Wild type; (e) *grk*; (f–h) *top*. (a) Blastoderm embryo. (b) At the onset of gastrulation, mesodermal cells (M) invaginate along the ventral side. The cephalic furrow (CF) becomes visible at 66% egg length. The posterior midgut (PMG) forms as a shallow curved plate at the posterior end of the embryo, directly underneath the pole cells. (c) During middle stages of gastrulation, the posterior midgut advances along the dorsal midline of the embryo, followed by the initially ventrally situated germ band (GB), which consists of ectoderm and mesoderm. The cells that originally occupied the dorsal-most region of the embryo become very thin and flexible and form the amnion-serosa (AS), which gives way to the advancing midgut and germ band by folding and moving more laterally. (d) The fully extended germ band stretches along the ventral side, around the posterior pole, and along the dorsal side to the cephalic furrow. The posterior midgut has invaginated. (e, f) In embryos from mutant mothers an enlarged mass of mesodermal cells invaginates on the ventral side. Note the double micropyle in the egg from *grk* (e). (g, h). The posterior midgut does not advance along the dorsal side of the embryo, and the posterior gut invagination occurs at a very posterior location. No germ band is present at the ventral side, due to the absence of ectoderm. Dorsally, no amnion-serosa is formed. Instead, a thickened layer of dorsal cells becomes apparent (DC). These dorsal cells resemble laterally derived ectodermal cells of the wild type and will form ventral cuticle at the end of embryogenesis. Magnification 120×.

pendent experiments were performed to obtain each of the relevant *top* mosaics. For the test of mutant germ cells in a wild-type soma, homozygous *top* germ-line clones were induced with gamma rays in heterozygous females, and *top* homozygous pole cells were transplanted into wild-type female hosts. In the first experiment ten irradiated females produced wild-type eggs and embryos from the homozygous mutant germ cells. In the second experiment ten wild-type host females gave rise to normal eggs and embryos that were derived from homozygous mutant germ cells (Table 1, series 4). For the reverse type of mosaics, wild-type germ cells derived from two different wild-type strains were transplanted into homozygous *top* hosts, whereby the second series was specially designed to allow clear identification of every single egg and embryo derived from implanted wildtype germ cells (Table 1, series 5 and 6). In both series only eggs and embryos of *top* phenotype were produced by the mosaic females (Figure 5). The *top* mutation therefore interferes with a process occurring in somatic cells—a process relevant not only for the proper patterning of the chorion, but also for the proper dorsoventral patterning of embryonic cells.

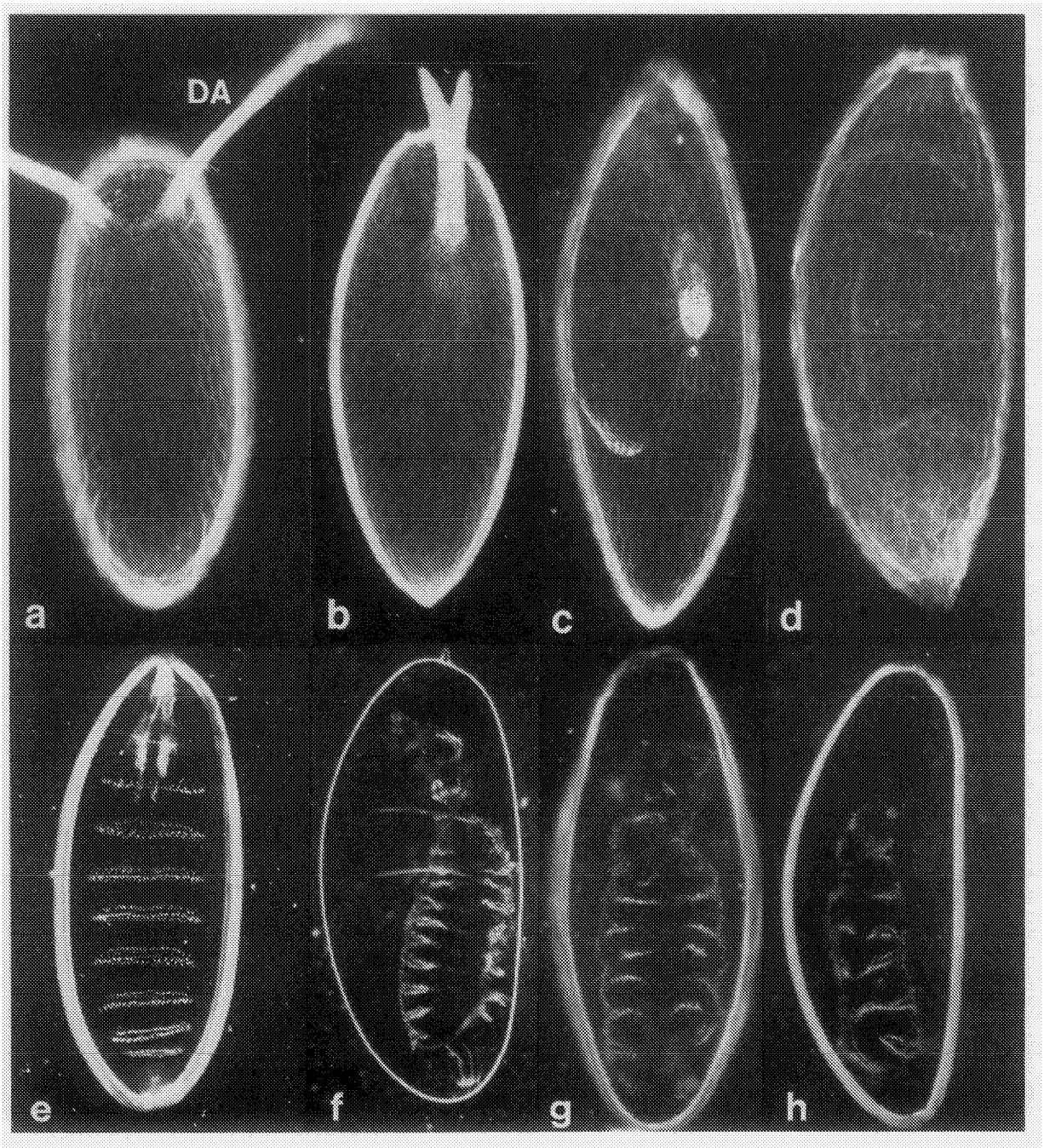

Figure 3. Dark-Field Micrographs of Chorions and Embryonic Cuticles inside Vitelline Membrane

(a, e) Wild type; (b, f) *top*; (c, d, g, h) *grk*. (a) The wild-type egg carries two dorsal appendages (DA) on its dorsal anterior side, their base located at 82% egg length (SD, 1.2%). The posterior end of the wild-type egg is rounded. (b) In the weak mutant phenotype the egg bears only one, fused dorsal appendage, inserted on the dorsal midline, at 70% egg length (SD, 3.8%). (c) In the intermediate mutant case the dorsal appendage is reduced to a knob of appendage material at 59% egg length (SD, 16.4%). The posterior end of the mutant egg is pointed, and the egg is longer than the wild-type egg. (d) In the strong mutant phenotype no dorsal appendage is formed at all. (e) The wild-type embryo forms characteristic bands of setae on the ventral third of its cuticle. (f) In the weak mutant phenotype a stripe of dorsal cuticle is flanked on either side by bands of ventral setae. The cuticle is usually not wide enough to surround the entire embryonic circumference. The head is very reduced, and usually only parts of the ventral plate and parts of the so called T-ribs can be distinguished. Filzkorper and spiracles are still visible at the posterior end of the embryo. (g) In the intermediate mutant phenotype progressively less dorsolateral cuticle separates the ventral bands of setae. The head is more reduced, and no spiracles and filzkoper are formed. (h) In the strong mutant phenotype only a narrow stripe of cuticle is formed along the dorsal midline. This cuticle carries bands of ventral denticles that are not separated by dorsal or lateral cuticle. No head or telson structures are visible. Magnification 145×.

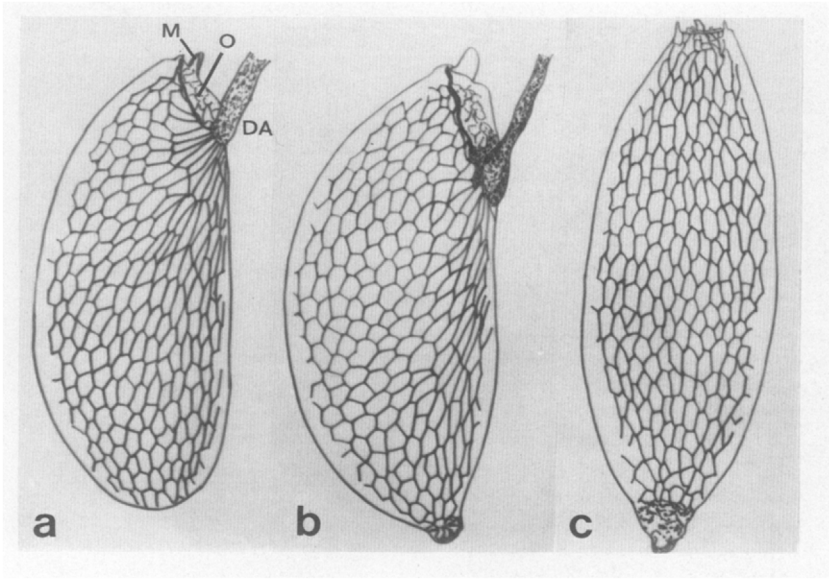

Figure 4. Chorion Phenotypes in Lateral View, with Follicle Cell Imprints Outlined

Camera lucida drawings, dorsal side to the right. (a) Wild type. (b) *top*. (c) *grk*. DA, dorsal appendage; M, micropyle; O, operculum. Note the difference in cell shape between the follicle cell imprints on the dorsal and ventral side of the wild-type egg in (a), where dorsal follicle cells are more elongated than ventral follicle cells. In the extreme mutant phenotype (c) all follicle cells are of similar shape. Cell counts made from such drawings indicate that in our sample the follicle cell population giving rise to the main body of the wild-type chorion is 623 (SE, 18.5). The cell population forming the main body in the weak chorion phenotype (b), with one fused dorsal appendage, amounts to 645 (SE, 35.3). The total cell population in the strong mutant phenotype (c), with no dorsal appendage, amounts to 806 (SE, 19.0). Magnification 115×.

Double-Mutant Phenotypes

Interdependence of genetic pathways can often be assessed by analyzing double-mutant animals (see, e.g., Beadle and Ephrussi, 1936; Baker and Ridge, 1980). Females were constructed that were homozygous for both *grk* and *top*. The eggs produced by such females always resembled the strong mutant cases in which most eggs lack dorsal appendages, have double micropyles, and are usually unfertilized. The phenotype of eggs and embryos was therefore essentially indistinguishable from the phenotype produced by the single mutations, except that the combination of intermediate *grk* with *top* resulted in a stronger phenotype than is usually produced by either mutation alone.

I also analyzed females that were homozygous for *fs(1)K10* and *grk* or *top*. *fs(1)K10* causes a phenotype apparently opposite to that of *grk* or *top*: it "dorsalizes" both the chorion and the embryos (Wieschaus et al., 1978; Wieschaus, 1979; Figure 6a). Eggs produced by *fs(1)K10* females possess enlarged dorsal appendages that ventrally surround the egg, and the eggs are shorter and more rounded. The embryos form mostly dorsal hypoderm, with the exception of the more posterior abdominal segments carrying narrow bands of ventral setae belts. None of these dorsalized mutant features is visible in the eggs or embryos produced by females homozygous for *fs(1)K10* and *grk* or for *fs(1)K10* and *top*. Such double-mutant females give rise to eggs and embryos that are in-

Table 1. Production of Germ-Line Mosaics by Pole Cell Transplantation

Series	Genotype of Donor Parents	Genotype of Host Parents	No. and Genotype of Surviving Host Females	No. of Mosaic Females	No. of Mosaic Females That Produced Phenotypic Wild-Type Eggs and Embryos, and Genotype of Implanted Germ Cells	No. of Mosaic Females That Produced Phenotypic *grk* or *top* Eggs and Embryos
1	♀ *grk*/+ × ♂ *grk*/*grk*	+/+	21 +/+	9	3 *grk*/+	**6**
2	♀ *grk*/*grk* × ♂ *grk*/*grk*	+/+	4 +/+	4	0	**4**
3	+/+	♀ *grk*/+ × ♂ *grk*/*grk*	13 *grk*/+ 9 *grk*/*grk*	4 4	4 +/+ **4** +/+	0 (4)[a]
4	♀ *top*/+ × ♂ *top*/*top*	+/+	46 +/+	19[b]	8 *top*/+ **10** *top*/*top*	0 0
5	+/+	♀ *top*/+ × ♂ *top*/*top*	28 *top*/+ 14 *top*/*top*	10 2	10 +/+ 0	0 **2**
6	+/+	♀ *top*/+ × ♂ *top*/*top*	16 *top*/+ *20* *top*/*top*	4 5	4 +/+ 0	0 **5**[c]

Genotypes are given only with respect to *grk* and *top*. For implanted germ cells, the genotypes were inferred from genetic markers displayed by adult progeny. Mosaicism was determined by histological staining of the ovaries of all surviving host females.

[a] These mosaic females laid *grk* eggs derived from their own germ line besides the wild-type eggs derived from the implanted germ cells.

[b] One of the mosaic females produced only unfertilized eggs of wild-type morphology.

[c] Due to the dominant female sterile mutation ovo^{D1} present in the host germ line, all of the eggs produced by these females derived from the implanted germ cells.

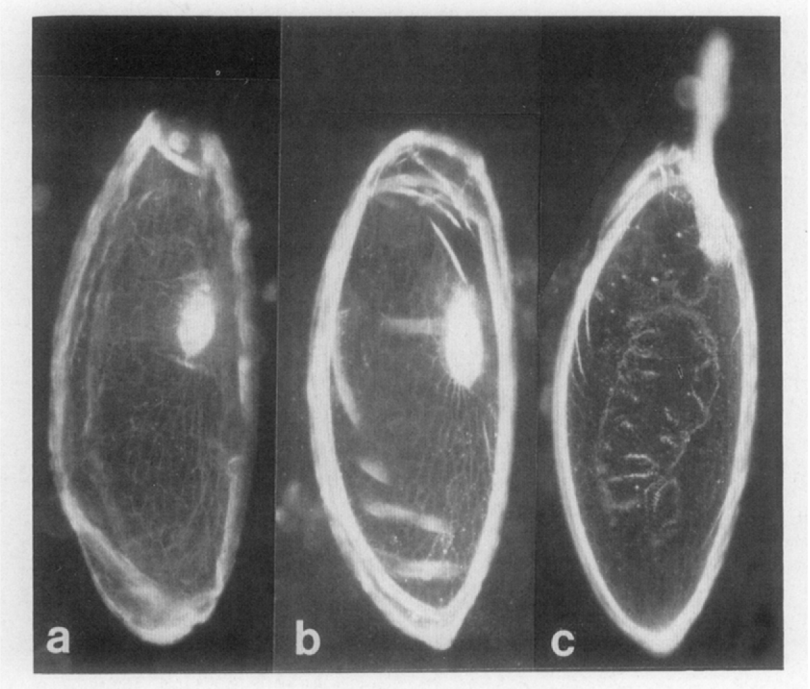

Figure 5. Chorion and Cuticle Phenotypes Produced by Females with Germ-Line Mosaicism

Dark-field micrographs. (a) Ventralized egg derived from an egg chamber where oocyte and nurse cells were homozygous mutant for *grk* but the follicle cells were *grk*$^+$. (b, c) Ventralized egg and embryo derived from egg chambers where oocyte and nurse cells were *top*$^+$ but the follicle cells were homozygous mutant for *top*. Magnification 110×.

distinguishable from those produced by single-mutant *grk* or *top* females (Figure 6b). *grk* and *top* are therefore epistatic over *fs(1)K10*, indicating that the wild-type *grk* and *top* genes are necessary to express the *fs(1)K10* mutant phenotype.

Females doubly mutant for *grk* and *dorsal* or for *dorsal* and *top* were also constructed. Mutations in *dorsal* lead to strong dorsalizations of the embryo but have no effect on egg morphology (Nüsslein-Volhard et al., 1980). The eggs produced by the double-mutant females still show the egg morphology characteristic of *grk* or *top*, but the embryos inside are completely dorsalized (Figure 6d). The *dorsal* wild-type product is therefore required for the expression of the *grk* or *top* mutant embryonic phenotype, but it is not required for the expression of the *grk* or *top* mutant egg morphology. This result rules out the possibility that the altered egg shape of *grk* and *top* in itself prevents the formation of dorsal embryonic structures inside such eggs, since dorsal embryonic structures are formed in the elongated eggs of the double-mutant females. In addition, the result argues that the *grk* and *top* mutations act via a different mechanism on follicle cells than on embryonic cells. The embryonic cells require *dorsal*$^+$ product to produce the ventralized *grk* and *top* phenotype, whereas the follicle cells do not, indicating that in embryos, but not in follicle cells, a process requiring *dorsal*$^+$ product intervenes between the action of *grk* and *top* and final dorsoventral determination.

Discussion

This paper describes two new genes that are required for the normal dorsoventral pattern of the Drosophila egg. The most striking features of the mutant phenotypes are the ventralization of the follicle cell epithelium situated on the outside of the egg chamber and the ventralization of the zygotic embryonic cells that form inside the fertilized egg.

This ventralization is most obvious in the embryos at gastrulation and is somewhat different from the ventralization of embryos produced by the dominant maternal-effect mutation *Toll*^{1} (Anderson et al., 1985a, 1985b). In *Toll*^{1}-derived embryos the mesoderm is not markedly increased, but rather the loss of dorsal structures is compensated by a drastic enlargement of the ventral hypoderm, which is characteristic of wild-type ventrolateral positions. In embryos derived from *grk* or *top* females, ventral hypoderm is also produced by cells situated in dorsal positions, but this hypoderm does not appear markedly increased in size. The major gain of cells in *grk* and *top* mutants is observed in the mesoderm. This could indicate a difference in the mechanism of embryonic ventralization between *Toll* and *grk* or *top*. Alternatively, it might reflect quantitative differences in ventralization, if the degree of ventralization determined not only which dorsal structures are deleted but also which ventral structures are predominantly expanded.

During the formation of the wild-type chorion, the two dorsal appendages and the operculum are formed by three populations of migrating follicle cells, each containing roughly 80–120 cells (Koch and King, 1966; King, 1970; Margaritis, 1985). These cells derive from a dorsal or dorsolateral location within the egg chamber. The cells that remain on the dorsal side of the egg chamber after the migration contribute to the main body of wild-type eggs. In wild-type eggs, they assume a stretched appearance, reflecting the smaller number of cells left on that side to span the distance between anterior and posterior ends. In the eggs produced by females mutant for *grk* or *top*, fewer cells contribute to dorsal appendage or operculum, whereas the population of follicle cells that give rise to the main body of the chorion is enlarged. In the extreme mu-

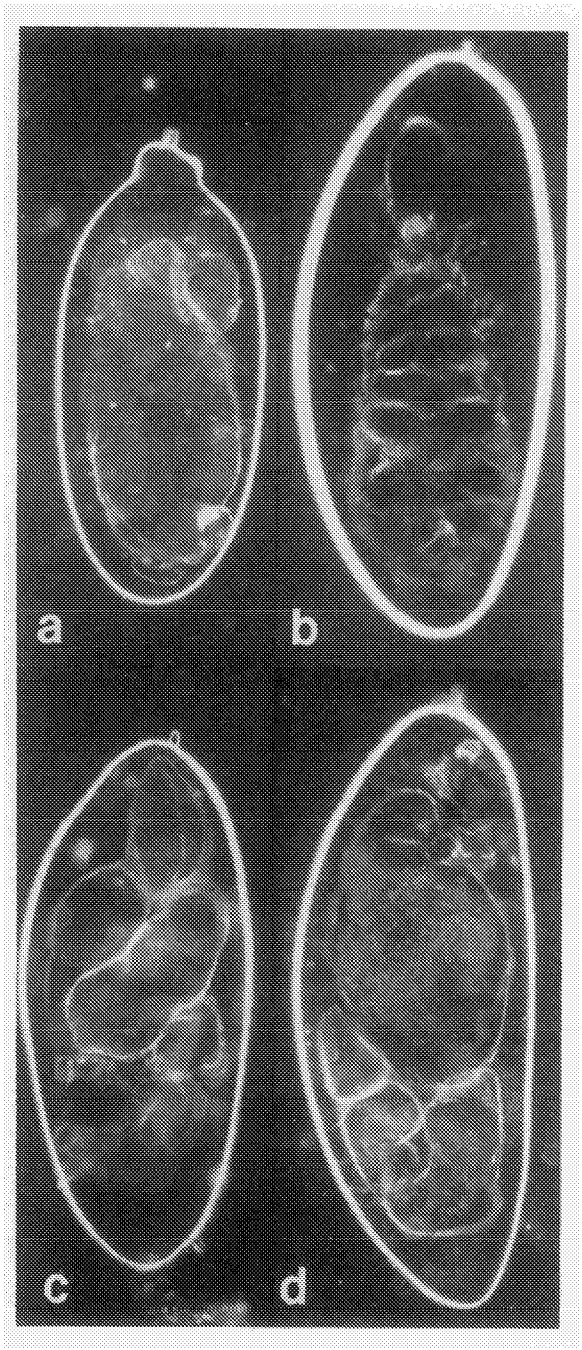

Figure 6. Double-Mutant Phenotypes

Dark-field micrographs of cuticles in vitelline membrane. (a) Embryo derived from a female homozygous for *fs(1)K10*. The *fs(1)K10* egg is shorter; the embryo is dorsalized and forms only dorsal cuticle. (b) Embryo derived from a female homozygous for *fs(1)K10* and *top*. The egg has the elongated (ventralized) *top* morphology. The embryo is ventralized and forms the characteristic stripe of ventral cuticle, similar to embryos derived from females homozygous for *top* alone. (c) Embryo derived from a female homozygous for *dorsal*. The egg is of wild-type morphology. The embryo is dorsalized and forms only dorsal cuticle. (d) Embryo derived from a female homozygous for *dorsal* and *top*. The chorion of this egg, which was removed for this picture, showed the characteristic *top* morphology. The embryo is dorsalized and is indistinguishable from an embryo derived from a female homozygous for *dorsal* alone.

tant egg chambers, where no cells migrate out of the epithelium to form appendages, none of the follicle cells assume the stretched appearance. The graded reduction in dorsal appendages and operculum argues that dorsal pattern elements are most sensitive to the mutations, whereas the increase in the main body of the chorion argues that the loss of dorsal structures is associated with a shift to other, more ventral cellular fates. This ventralization of the follicle cell epithelium was correlated with the ventralization seen in the embryo: inside eggs with weaker chorion phenotypes the ventralization of the embryos tended to be of weak or intermediate phenotype, whereas inside the eggs with intermediate or strong chorion phenotypes the ventralization of the embryos was always intermediate or strong.

In addition to the ventralization, a small and variable alteration of the final anterior-posterior chorion pattern is also observed. A second micropyle is often formed at the posterior pole of extreme *grk* eggs, and the reduced dorsal appendage of *grk* or *top* is usually shifted posteriorly. This pattern alteration of the chorion is at present difficult to interpret, particularly since no such alteration of anterior-posterior fates was visible in the embryos.

The epistatic relationship of *grk* and *top* over *fs(1)K10* can be explained if these three genes participate in a common pathway or in a linear series of events. In most respects, *fs(1)K10* causes the opposite effect of *grk* and *top*. *fs(1)K10* eggs have dramatically enlarged dorsal appendages, they are shorter and rounder than normal eggs, and they give rise to dorsalized embryos. Given the similarity in the array of effects in the mutant phenotype, we think it likely that these three genes interact directly and alter a single process occurring during oogenesis, rather than participate in different processes that are not directly related but occur in a linear series, a situation that could also lead to an epistatic relationship (see below).

The epistasis observed in the double-mutant combinations between *grk*, *top*, and *fs(1)K10* with *dorsal* might also be explained in a model where these genes directly regulate the *dorsal* gene or any of the genes in the *dorsal–Toll* group of genes. However, given the differences in mutant phenotype, it appears more likely that *grk*, *top*, and *fs(1)K10* are involved in different processes than the genes of the *dorsal* group. The *grk*-, *top*-, and *fs(1)K10*-dependent processes might establish or maintain the asymmetries of the egg chamber during oogenesis alone. The immediate consequence of these patterning processes would be evident in the pattern of the chorion as secreted by the follicle cells. At the same time these patterning processes would indirectly control the performance of the later-acting genes of the *dorsal* group—for instance, by distributing "sources" or by regulating diffusion conditions within the egg. The embryonic phenotype caused by mutations in *grk*, *top*, and *fs(1)K10* would, according to this view, also be dependent on the presence or absence of the *dorsal* genes, as was indeed seen in the double combinations with the mutation *dorsal*.

At the level of the present analysis, the mutant phenotypes of *grk* and *top* seemed identical, both with respect to the chorion as well as the embryo. It was therefore sur-

prising to find that the *grk* mutations act exclusively in the germ line, yet the *top* mutation interferes exclusively with processes occurring in somatic cells. Other maternal-effect genes, including *fs(1)K10*, that alter the pattern of the embryo have been tested for germ-line versus somatic requirements; so far, all except *dic*, which at low frequency alters the anterior-posterior arrangement of nurse cells and oocyte (Frey and Gutzeit, 1986), have been shown to be germ-line-dependent (Wieschaus et al., 1978; Perrimon and Gans, 1983; Lehmann and Nüsslein-Volhard, 1986; Schüpbach and Wieschaus, 1986b). Thus it might have seemed that in Drosophila the asymmetries present in the mature egg are established by processes that require gene expression only within the oocyte and its sister nurse cells. Given the germ-line dependence of the altered chorion pattern in *fs(1)K10* and *grk*, some form of spatial information must be passed from the cells of the germ line to the follicle cells. Both *fs(1)K10* and *grk* are involved in the correct production or distribution of this information. The somatic dependence of *top* might initially have suggested that this mutation prevents the follicle cells from perceiving this spatial information generated in the germ line. This is, however, less likely, given that *top* also alters the pattern of the embryonic cells. Whatever somatic process *top* interferes with during oogenesis, this process is also of consequence for embryonic patterning. Some somatic cells therefore must be involved in the distribution of pattern information inside the oocyte. *top* is epistatic over *fs(1)K10*. The *fs(1)K10* phenotype, which is caused by an alteration in the germ line, is therefore only produced in the absence of the *top* mutation in the soma. Consequently, the dorsoventral patterns of the egg and embryo are dependent on close interactions between somatic cells and germ cells, where alteration of one cell type can cause the corresponding alteration of the other.

Experimental Procedures

Genetics

Six alleles of *grk* and one allele of *top* were isolated in ethyl methanesulfonate screens designed to identify female sterile mutations on the second chromosome of Drosophila melanogaster (Schüpbach and Wieschaus, unpublished). The mutant alleles were genetically mapped by standard methods; *grk* was found to lie at meiotic position 2-30 and *top* at meiotic position 2-100. Females homozygous for the alleles grk^{HK} and grk^{HL} and grk^{DC} produce eggs and embryos of the intermediate and strong phenotypes only. Females homozygous for grk^{WG} and grk^{QI} produce eggs and embryos of predominantly intermediate phenotypes, but even from single females eggs and embryos can vary from weak to strong phenotypes. Females homozygous for grk^{HG} and top^{1} produce eggs and embryos predominantly of weak and intermediate mutant phenotypes.

Characterization of Mutant Phenotypes

For microscopic inspection of chorions and embryonic cuticular phenotypes, eggs were processed as previously described (Wieschaus and Nüsslein-Volhard, 1986). For analysis of gastrulation, the mutations grk^{WG}, grk^{HK}, and top^{1} were crossed into a strain carrying the maternal-effect mutation *klarsicht* on the third chromosome. *klarsicht* enhances the visibility of embryonic cells formed inside the egg without affecting normal development (Wieschaus and Nüsslein-Volhard, 1986). Videotapes of living embryos were made with a Panasonic NV-8050 time-lapse video recorder. For histological sections embryos were processed as previously described (Eichenberger-Glinz, 1979; Schüpbach and Wieschaus, 1986a).

Counts of follicle cell imprints were made on camera lucida drawings of chorions viewed in a Zeiss light microscope. Between eight and twelve eggs from wild-type, *top*, or *grk* homozygous females were drawn in dorsal, ventral, or lateral view. Imprints were counted in longitudinal sectors, corresponding to one-sixth of the egg circumference, and for each genotype an average number of imprints per sector was calculated from at least four dorsal sectors, four ventral sectors, four dorsolateral sectors, and four ventrolateral sectors. The total average number of follicle cell imprints per genotype was then estimated by adding the means of one average ventral, one average dorsal, two average dorsolateral, and two average ventrolateral sectors.

Pole Cell Transplantation

Pole cells were transplanted according to the method of Van Deusen (1976). Six different series of transplantation were carried out (Table 1). Appropriate visible genetic-marker mutations allowed the identification of the genotype of transplanted pole cells in all cases where the transplanted pole cells gave rise to adult progeny. To identify the cases where the implanted pole cells did not give rise to normal eggs and offspring, two additional marker mutations were used. In all transplantations either the donors (series 3 and 5) or hosts (series 1, 2, 4, and 6) were homozygous for the histological marker *mal* on their X chromosomes. Successfully implanted germ cells were therefore always identified by staining the ovaries of the adult hosts for aldehyde oxidase activity (Janning, 1972; Marsh and Wieschaus, 1977). In series 1, 2, 4, and 6, the female hosts carried also the ovo^{D1} mutation. ovo^{D1} is strictly germ-line-dependent and leads to atrophied adult ovaries that contain only a few small egg chambers (Busson et al., 1983; Perrimon and Gans, 1983). After successful implantation of wild-type germ cells, these females will produce eggs and embryos that are exclusively derived from the implanted germ cells. To mark the X chromosome that carried ovo^{D1} with the marker mal, I mutagenized males carrying the original ovo^{D1} v^{24} chromosome, and established a line from an F1 male that had a newly induced *mal* mutation (designated mal^{tr}) on the original ovo^{D1} v^{24} chromosome (data not shown).

Mitotic Recombination

For the induction of germ-line mosaicism by irradiation of heterozygous germ cells, the dominant female-sterile technique was employed (Wieschaus, 1980; Schüpbach, 1982). The *top* chromosome, marked with *cn* and *bw*, was brought in *trans* to a chromosome that carried the dominant female-sterile mutation *Fs(2)D*, and the heterozygous individuals were irradiated as second instar larvae (40 + 6 hr) with approximately 1300 gamma rays. Clones of germ cells that had lost *Fs(2)D* could be detected as females that laid fertilized eggs. To demonstrate that the wild-type eggs obtained from *Fs(2)D/top* females were derived from homozygous *top* clones, all the resultant progeny were tested for the presence of a maternally derived *top* allele.

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