cappuccino and *spire*: two unique maternal-effect loci required for both the anteroposterior and dorsoventral patterns of the *Drosophila* embryo

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cappuccino and *spire* are unique *Drosophila* maternal-effect loci that participate in pattern formation in both the anteroposterior and dorsoventral axes of the early embryo. Mutant females produce embryos lacking pole cells, polar granules, and normal abdominal segmentation. They share these defects with the posterior group of maternal-effect genes. Although embryos are defective in abdominal segmentation, in double mutant combinations with *Bicaudal D*, abdominal segments can be formed in the anterior half of the egg. This indicates that embryos produced by mutant females contain the 'posterior determinant' required for abdominal segmentation (Nüsslein-Volhard et al. 1987) and suggests that the wild-type gene products are not required for production of the posterior determinant but, rather, for its localization or stabilization. The *vasa* protein, a component of polar granules, is not localized at the posterior pole of mutant egg chambers or embryos, providing additional support for the hypothesis that localization to or stabilization of substances at the posterior pole of the egg chamber is defective in mutant females. Females mutant for the strongest alleles also produce dorsalized embryos. Phenotypic analysis reveals that these dorsalized embryos also have abdominal segmentation defects. The mutant phenotypes can be ordered in a series of increasing severity. Pole cell formation is most sensitive to loss of functional gene products, followed by abdominal segmentation, whereas normal dorsoventral patterning is the least sensitive to loss of functional gene products. In addition, mutant females contain egg chambers that appear to be dorsalized, resulting in the production of eggs with dorsalized eggshells. Germ-line mosaics indicate that *cappuccino* and *spire* are required in the oocyte-nurse cell complex. This suggests that the eggshell phenotype results from altered pattern in the underlying germ cell. Also, we defined the epistatic relationships between several early patterning loci, on the basis of an analysis of the eggs and embryos produced by females doubly mutant for *cappuccino* or *spire* and other loci that affect the pattern of both the egg and the embryo. On the basis of our current knowledge of the genes involved in this process, we formulated a working model for the early steps in dorsoventral patterning.

[Key Words: *Drosophila*; *cappuccino*; *spire*; maternal effect; *grandchildless-knirps*; oogenesis]

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Both the eggshell and embryo of *Drosophila* are asymmetric. The egg possesses a flat dorsal surface, a rounded ventral surface, a micropyle and operculum at the anterior end, and two chorionic appendages located antero-dorsally. These asymmetric features of the egg become visible during the later stages of oogenesis. Earlier in oogenesis, the follicle cells, which surround the developing oocyte and secrete the eggshell, show an asymmetric distribution along the dorsoventral axis with respect to cell shape and density (King 1970). Within the embryo, the pattern of early zygotic gene expression is asymmetric both in the anteroposterior and dorsoventral axes. Localized gene expression along the anteroposterior axis of the embryo results in the formation of a series of segmentally repeated units with the head at one end and the telson at the other (for reviews, see Scott

and O'Farrell 1986; Akam 1987; Ingham 1988). Along the embryonic dorsoventral axis, asymmetric gene expression leads to the formation of the mesoderm ventrally with the neurogenic ectoderm, the dorsal ectoderm, and the amnioserosa forming progressively more dorsally (Doyle et al. 1986; St. Johnston and Gelbart 1987; Steward et al. 1988; Thisse et al. 1988; for reviews, see Anderson 1987; Levine 1988).

The asymmetries observed in eggs and embryos are specified genetically. Numerous maternal-effect loci have been identified that function in the production of either the dorsoventral or anteroposterior patterns of the embryo. The maternal-effect loci required for patterning in the anteroposterior axis of the embryo can be divided into three groups (for review, see Nüsslein-Volhard et al. 1987). The first group is necessary for the establishment

of the anterior pattern of the embryo, including the acron, head, and thorax (Frohnhofer and Nüsslein-Volhard 1986, 1987; Driever and Nüsslein-Volhard 1988a,b). The second group, known as the *grandchildless-knirps* (Schüpbach and Wieschaus 1986) or posterior group, is required for the pattern in the posterior half of the embryo, including posterior pole plasm and abdominal segmentation (Boswell and Mahowald 1985; Lehmann and Nüsslein-Volhard 1986, 1987; Schüpbach and Wieschaus 1986). The third group acts to specify the pattern at both ends of the embryo, namely the acron anteriorly and the telson posteriorly (Degelman et al. 1986; Schüpbach and Wieschaus 1986; Klingler et al. 1988; Strecker et al. 1989). In the dorsoventral axis, most of the loci identified are required to establish ventral cell fates, so that 'dorsalization' occurs in loss-of-function mutations, with embryos lacking structures derived from the more ventrally specified mesoderm and neurogenic ectoderm (for review, see Anderson 1987). Because all of the reported maternal-effect loci that affect pattern formation are involved in generating either the dorsoventral or anteroposterior axis, but not both, these two axes of the embryo seem to be specified independently of one another.

A small group of loci have been identified that produce similar pattern alterations in both the eggshell and embryo, when mutated. This allows the pattern of the embryo to be related to cellular patterns and spatial organization already visible during oogenesis. This relationship is intriguing because the eggshell and embryo are made by two very different cell types. The eggshell is secreted by somatically derived follicle cells, whereas the embryo develops inside the germ-line-derived egg cytoplasm. Females mutant for *fs(1)K10* produce almost symmetrically shaped eggs with dorsal appendage material secreted around the entire anterior end in a ring (Wieschaus et al. 1978; Wieschaus 1979, 1980; Wieschaus and Szabad 1979). During oogenesis, the developing oocyte and the surrounding follicle cells form a dorsalized egg chamber. Embryos developing inside *fs(1)K10* eggs are dorsalized also. *torpedo* (*top*) and *gurken* (*grk*) (Schüpbach 1987) are maternal-effect loci with the opposite effects from *fs(1)K10* on egg and embryonic patterns. Mutant mothers produce elongated eggs with little or no dorsal appendage material (corresponding to ventralization during oogenesis) and ventralized embryos. The correspondence between pattern defects of the eggs and embryos from *fs(1)K10*, *top*, and *grk* mutant mothers indicates a relationship between the genetically defined asymmetries of oogenesis and embryogenesis.

In this paper we describe two additional maternal-effect loci, *cappuccino* (*capu*) and *spire* (*spir*), that are required for patterning during both oogenesis and embryogenesis. Mutations in these loci result in the production of *fs(1)K10*-like eggs containing dorsalized embryos. A striking feature of both loci is that mutant embryos also contain very specific alterations in the anteroposterior axis. These defects closely resemble those caused by mutations in the *grandchildless-knirps* or posterior

group of maternal-effect loci. *capu* and *spir* are therefore the first loci known to be involved in patterning in both the dorsoventral and anteroposterior axes of the embryo. Their existence indicates that the dorsoventral and anteroposterior patterning processes share some common features.

Results

Twelve alleles of *spir* (2–54) and five alleles of *capu* (2–8) were isolated in several screens for female sterile mutations. The alleles can be placed in a phenotypic series. Females mutant for weaker alleles produce embryos lacking pole cells, polar granules, and normal abdominal segmentation. Females mutant for stronger alleles produce dorsalized eggshells and embryos (see Table 1).

Embryos produced by capu or spir mutant females resemble those produced by the posterior or grandchildless-knirps group of maternal-effect mutants

Females mutant for *capu* or *spir* produce embryos lacking pole cells, posterior pole plasm, and normal abdominal segmentation, defects typical of the posterior or *grandchildless-knirps* class of maternal-effect mutants. Pole cells, the progenitor cells of the germ line (Mahowald et al. 1979), which form at the posterior pole of a normal embryo, are absent in all embryos laid by mutant females (see Fig. 1). A small fraction of the offspring of females homozygous for the weakest alleles (e.g., *capu^{HK3}*, *spir^{A1}*) survive to adulthood. Examination of the gonads dissected from these adults indicates that they are agametic, confirming that this aspect of the mutant phenotype is 100% penetrant.

Mutant embryos are also missing polar granules, electron-dense ribonucleoprotein structures normally found in the posterior cytoplasm of wild-type embryos, thought to be important in specification of the germ line (Mahowald et al. 1979). Electron micrographs of sections from the posterior pole of early embryos from *capu^{HK3}*, *spir^{RP}* homozygous mutant mothers failed to reveal the presence of any polar granules, although polar granules were observed in sections of wild-type embryos of the same developmental stage (see Fig. 1). In addition, mutant embryos and egg chambers do not accumulate the *vasa* protein, a component of the polar granules, at the posterior pole. In wild-type females, the *vasa* protein is seen first at the posterior pole during oogenesis in stage-10 egg chambers and becomes incorporated into the pole cells during embryogenesis (Hay et al. 1988a,b). To visualize the distribution of the *vasa* protein in mutant embryos, embryos derived from *capu^{G7}*/*Df(2L)ed^{SZ1,2}* and homozygous *spir^{RP}* females were immunostained with a monoclonal antibody that reacts with *vasa* protein (mAb46F11, generously provided by Bruce Hay). Mutant embryos do not exhibit concentrated staining in the posterior pole (Fig. 1). To determine whether the *vasa* protein is localized initially to the posterior pole during oogenesis, ovaries from *capu^{G7}*/*Df(2L)ed^{SZ1,2}* and *spir^{RP}* females were sectioned

Table 1. Percentages of eggshell and cuticle phenotypes of unhatched eggs produced by females of various mutant genotypes

Eggshell phenotype (%)			Cuticle phenotype (%)				Miscellaneous (%)
			unfertilized	dorsalized ^a		<i>knirps</i> ^b (%)	
<i>capu</i>							
HK3/HK3	normal	86	22	10		68	0
	abnormal	14	79	15	(+ 57 = 72)	6	0
HK3/Df	normal	55	25	22		53	0
	abnormal	45	69	28	(+ 44 = 72)	3	0
RK/RK	normal	55	45	11		45	0
	abnormal	45	72	14	(+ 27 = 41)	14	1
RK/Df	normal	27	12	29		59	0
	abnormal	73	77	15	(+ 65 = 80)	8	0.4
G7/G7	normal	14	45	0		55	2
	abnormal	86	72	5	(+ 27 = 32)	20	0
G7/Df	normal	30	38	16		42	1
	abnormal	70	46	19	(+ 8 = 27)	31	4
<i>spir</i>							
41/41	normal	95	33	40		27	0
	abnormal	5	58	42	(+ 25 = 67)	0	0
41/Df	normal	33	55	8		33	1
	abnormal	67	74	18	(+ 19 = 37)	8	0
03/03	normal	66	41	18		40	1
	abnormal	34	67	20	(+ 26 = 46)	13	0
03/Df	normal	32	37	9		54	0
	abnormal	68	69	15	(+ 36 = 51)	16	0
RP/RP	normal	41	46	11		42	1
	abnormal	59	53	30	(+ 7 = 37)	17	0
RP/Df	normal	35	39	19		42	0
	abnormal	65	74	14	(+ 35 = 49)	12	0

Larvae hatch from a small percentage of eggs laid by mutant females. The percentages of the total eggs laid that hatch are as follows: *capu*^{HK3}, 15%; *capu*^{RK12}, 2%; *capu*^{G7}, 1%; *spir*⁴¹, 8%; *spir*⁰³, 3%; and *spir*^{RP}, 4%.

^a In the case of the strong chorion phenotype, there is often a substantial increase in the number of unfertilized eggs as compared to the normal eggs produced by the same females. When fertilized, such strongly dorsalized eggs would show the dorsalized cuticle phenotype. Therefore, the difference between the percentage of unfertilized eggs in the normal and abnormal categories should be added to the percentage of dorsalized eggs (numbers in parentheses).

^b Some of these *knirps*-type embryos have head defects.

and stained with the antibody. We did not observe staining in the posterior pole of egg chambers from either mutant, although wild-type sections on the same slides were labeled clearly by the antibody (data not shown). This suggests *vasa* protein is not localized correctly at the posterior pole of mutant egg chambers during oogenesis.

Females homozygous for *capu* and *spir* produce embryos with abdominal segmentation defects similar to those produced by mutations in the *grandchildless-knirps* or posterior class of maternal-effect loci (Boswell and Mahowald 1985; Lehmann and Nüsslein-Volhard 1986, 1987; Schüpbach and Wieschaus 1986). These embryos have relatively normal heads and thoraces but show abdominal segmentation defects ranging from fusion of neighboring segments to deletion of all of the abdominal segments (see Fig. 2B). The severity of abdominal defects varies between different alleles. For example, in the weak allele *capu*^{HK3}, a large fraction of embryos have only minor abdominal segmentation defects, whereas in *capu*^{RK}, most embryos are entirely lacking abdominal segments. Moreover, when a weak or intermediate allele is tested in trans to a chromosomal deficiency, the frequency of abdominal defects increases,

indicating that the severity of the defects is correlated to the amount of gene product present (see Table 1). In mutant embryos the expression pattern of *fushi tarazu* (*ftz*), a gene that normally is expressed in seven transverse stripes at the cellular blastoderm stage (Hafen et al. 1984; Carroll and Scott 1985), is similar to that produced by mutants in other members of the *grandchildless-knirps* group of loci (Carroll et al. 1986). To simplify the task of visualizing *ftz* expression, male carrying a *ftz-lacZ* gene fusion [kindly provided by Yash Hiromi (Hiromi et al. 1985)] were mated with *capu*^{RK} and *spir*⁰³ homozygous females, and the β -galactosidase pattern in the mutant offspring was visualized using immunostaining. The *ftz-lacZ* pattern is abnormal in almost all *capu*^{RK} and *spir*⁰³ mutant embryos. In *spir*⁰³ most of the embryos have only four stripes, instead of the normal seven. The anterior two stripes are of relatively normal spacing, width, and intensity, followed by a broad faint third stripe and a fourth normal stripe (see Fig. 3). Some of the *capu*^{RK} mutant embryos have a *ftz* pattern similar to *spir*⁰³, although some have additional stripes in the region between the second and posterior-most stripe, similar to the weaker mutations in the posterior group genes (Carroll et al. 1986; Lehmann 1988).

Figure 1. The *grandchildless* phenotype: Embryos from mutant mothers lack pole cells, polar granules, and localized *vasa* protein. (A, C, and E) Embryos undergoing cellularization that have been immunostained with a monoclonal antibody that reacts with the *vasa* protein. Wild-type embryos (A) have pole cells and *vasa* staining at the very posterior end (arrow), whereas embryos from *capu* (C) and *spir* (E) females lack pole cells and localized *vasa* staining. (B, D, and F) Polar granules are present in electron micrographs of sections from the posterior end of a wild-type embryo (B; arrow), but absent in sections from *capu* (D) and *spir* (F) embryos. Genotype of mother: (A and B) Wild-type; (C) *capu*^{G7/Df}; (D) *capu*^{HK3/capu}^{HK3}; (E and F) *spir*^{RP/spir}^{RP}. Anterior is to the left and dorsal is up.

capu and *spir* mutant egg chambers contain the 'posterior activity' required for abdominal segmentation

The various members of the posterior or *grandchildless-knirps* group of loci show striking differences in their effects on the bicaudal pattern produced by *Bicaudal D* (*Bic-D*). *Bic-D* is a dominant maternal-effect mutation that produces symmetrical bicaudal embryos (Mohler and Wieschaus 1985, 1986). Some members of the posterior group in combination with *Bic-D* suppress the formation of an anterior abdomen, whereas others do not (Lehmann 1985; Lehmann and Nüsslein-Volhard 1986; R. Lehmann, pers. comm.). Therefore, we examined the phenotype of embryos produced by females homozygous for *capu*^{RK} or *spir*^{RP} and heterozygous for *Bic-D*^{71.34}. A number of different types of embryos are produced, in keeping with the variability of *capu*, *spir*, and *Bic-D*. In phenotypically bicaudal embryos, the posterior half of

the embryo (i.e., that with normal polarity) shows reduction in segmental pattern typical of *capu* or *spir*, whereas the anterior half (i.e., that with reverse polarity) shows normal segmentation (see Fig. 4). This indicates that mutations in *capu* and *spir* do not absolutely prevent the formation of abdominal segments but specifically interfere with abdominal segmentation in the posterior half of the embryo.

Some embryos produced by capu or spir mutant females resemble those produced by the dorsalizing class of maternal-effect mutants

Females homozygous or hemizygous for the stronger alleles of *capu* and *spir* produce dorsalized embryos (Table 1). The dorsalized phenotype can be seen in the cuticle at the end of embryogenesis (see Fig. 2C). The cuticle is a twisted tube with no denticle bands present on the ventral surface. The first evidence of the dorsalized embryo-

Figure 2. Embryos from mutant mothers produce cuticle resembling that of the *grandchildless-knirps* or dorsalizing classes of maternal-effect loci. (A) Wild-type cuticle; (B and D) *grandchildless-knirps*-type cuticles typical of *spir* or *capu* (B) and from *tudor* (D), a member of the *grandchildless-knirps* group of loci (Boswell and Mahowald 1985; Schüpbach and Wieschaus 1986); (C and E) dorsalized cuticles typical of *capu* or *spir* (C) and from *dorsal* (E). Genotypes of mothers: (B) *spir*⁰³; (C) *capu*^{RK}; (D) *tud*^{WC8}; (E) *In(2L)dl*^H/*Df(2L)TW119*. (fk) Filzkörper; (fkp) filzkörper patch.

Figure 4. Females doubly mutant for *Bic-D* and *capu* or *spir* are able to produce embryos with normal abdominal segmentation in the anterior of a bicaudal embryo, indicating that *capu* and *spir* embryos possess the posterior determinant. (A) Cuticle of a symmetrical bicaudal embryo. (B) Cuticle of a bicaudal embryo from a *spir*⁰³ *Bic-D*^{71.34}/*spir*^{RP} female. This embryo exhibits normal abdominal segmentation with reversed polarity in the anterior of the egg [marked by the micropyle (m)] but shows the abdominal segment deletions and filzkörper patches (fkp) (in an underlying focal plane), typical of *spir* in the posterior of the egg. (fk) Filzkörper. Large arrows indicate the polarity of the segments from anterior to posterior.

onic phenotype can be seen during gastrulation (e.g., Figs. 3 and 5). In many of the embryos derived from females homozygous for the strongest alleles, the ventral furrow is reduced or absent. Abnormal folds appear on the dorsal and ventral surfaces as the germ band fails to extend dorsally. In fixed embryos, it is possible to see that the cephalic furrow is more perpendicular to the longitudinal axis of the embryo than in wild type. These gastrula resemble those produced by other members of the dorsalizing class of maternal-effect loci, although *capu* and *spir* dorsalized gastrula have a reduced number of folds in the abdominal region.

Figure 3. Embryos from *capu* and *spir* mutant mothers exhibit altered *ftz* expression, similar to that observed in the *grandchildless-knirps* class of loci. Wild-type (A) embryos have seven stripes of *ftz* expression, as do dorsalized embryos produced by members of the *dorsal* group of maternal-effect loci (Carroll et al. 1987) and *fs(1)K10* (B) *capu*^{RK} females (C and D) and *spir*⁰³ (E and F) females produce embryos with a reduced number of *ftz* stripes. Typically, the anterior two stripes are normal. More posterior is a broad, sometimes faint, stripe and a posterior stripe of normal width. Embryos in C, E, and F are dorsalized to various extents.

Figure 5. Immunostaining with antibodies against *zen* and *twist* protein indicates that some embryos from mutant mothers are dorsalized. (A and B) Embryos stained with an antibody directed against *zen* protein. (A) Wild type express *zen* dorsally. (B) An embryo from *capu*^{RK} that exhibits expansion of *zen* staining around the ventral circumference as is seen in the dorsalizing class of loci (Rushlow et al. 1987). Similar expansions of *zen* staining are observed in *spir* mutant embryos. (C–G) Embryos stained with an antibody directed against *twist* protein. (C and D) Wild type showing *twist* staining in a ventral strip of cells; (E–G) Dorsalized embryos from *capu*^{G7/Df} (E and F) and *spir*^{RP} (G). Note the absence of staining in the central regions of these mutant embryos.

To visualize the extent of dorsalization, embryos derived from *capu* and *spir* mothers were stained with antibodies to the *zerknüllt* (*zen*) [generously provided by Chris Rushlow (Rushlow et al. 1987)] and *twist* [generously provided by Siegfried Roth (Thisse et al. 1988)] gene products. In wild-type embryos, *zen* protein is found first in embryos undergoing the thirteenth nuclear division in nuclei positioned at the surface of the most dorsal 40% of the embryo and at the anterior and posterior poles of the embryo. As development proceeds, *zen* protein becomes restricted to the dorsal side so that it is restricted to a dorsal strip 5–6 cells wide by gastrulation (Rushlow et al. 1987). Some *capu* and *spir* embryos show an expansion of *zen* ventrally (see Fig. 5), similar to the *zen* expression pattern in other dorsalizing mutants (Rushlow et al. 1987). However, in contrast to the other dorsalizing mutants, *zen* staining is not expanded uniformly along the anteroposterior axis but shows greater expansion of staining anteriorly than posteriorly, indicating a stronger dorsalization of more anterior regions of the embryo. In older *capu* and *spir* dorsalized embryos, the *zen* staining is much fainter than in wild-type controls. In wild-type embryos, *twist* protein is found

immediately prior to gastrulation in a midventral strip of cells ~12 cells wide (Thisse et al. 1988). During gastrulation, these cells invaginate to form the ventral furrow. In some *capu* and *spir* mutant embryos, *twist* staining is present only in ventral cells at the anterior and posterior poles of the embryo; ventral cells in the more central regions of the anteroposterior axis do not stain (see Fig. 5). Embryos that do stain with the *twist* antibody in the ventral furrow region often show a band of staining that varies in width along the anteroposterior axis.

The dorsalized *capu* and *spir* embryos also exhibit *grandchildless-knirps*-type pattern defects. All embryos derived from mutant mothers, including the dorsalized *capu* and *spir* embryos, lack pole cells. This lack of pole cells is atypical of mutations in the dorsalizing class of loci (Nüsslein-Volhard 1979) but is characteristic of the *grandchildless-knirps* class of loci (Boswell and Mahowald 1985; Lehmann and Nüsslein-Volhard 1986, 1987; Schüpbach and Wieschaus 1986). In embryos with a dorsalized cuticle phenotype, it is difficult to observe the anteroposterior pattern because the ventral denticles that mark the segment pattern are missing. To visualize

the anteroposterior pattern in embryos with a dorsalized phenotype, the expression of pattern of *ftz* was analyzed in the mutant embryos, as described earlier. In embryos that were classified as being dorsalized on the basis of their pattern of transverse folds at gastrulation, the number of *ftz-lacZ* stripes is reduced (see Fig. 3C, E, and F). This is not typical of dorsalized embryos produced by the dorsal group of maternal-effect loci (Carroll et al. 1987; this study Fig. 3B, *fs(1)K10*) and confirms that even dorsalized *capu* and *spir* embryos have abdominal segment deletions.

capu and *spir* participate in dorsoventral patterning during oogenesis

Females homozygous for the strongest alleles of *capu* or *spir* produce egg chambers and eggs that appear to be dorsalized. The functional unit of oogenesis is the egg chamber, a cellular complex that consists of the developing oocyte and 15 nurse cells surrounded by a monolayer of follicle cells, which secrete the eggshell. In wild-type egg chambers at stage 11, the follicle cells that are in contact with the dorsal, anterior side of the oocyte are distinguished easily from those on the ventral side because the dorsal follicle cells are more columnar than those on the ventral side (King 1970). In contrast, egg chambers produced by mutant females are symmetric; both the dorsal and ventral sides of the anterior oocyte are surrounded by columnar follicle cells, indicating that the egg chamber is dorsalized (see Fig. 6).

The dorsalization of the egg chamber during oogenesis is reflected in the eggshell of *capu* and *spir* mutant eggs.

Eggs from females homozygous for the strongest alleles frequently are dorsalized (see Table 1). Whereas two distinct dorsal appendages are formed at an anterodorsal location in wild type, the dorsal appendages often are fused dorsally and/or extended ventrally around the anterior circumference in the mutant (see Fig. 7). In addition, the shapes of the follicle cell imprints in the main body of the eggshell, which are elongated on the dorsal side and regularly shaped on the ventral side in wild-type eggs, do not show a dorsoventral difference in mutant eggs. The number of follicle cell imprints on the main body of the chorion is rather constant in normal eggs. In the dorsalized eggshells, the number of follicle cell imprints in the main body of the egg is reduced. Within the area of the eggshell that was analyzed (see Materials and methods), 378 ± 24 follicle cells secrete main body chorion in wild type, whereas in the severely dorsalized eggs, ~ 280 (*capu*, 288 ± 21 ; *spir*, 276 ± 33) follicle cells are secreting main body chorion (see Table 2). Because the number of follicle cells secreting the main body of the eggshell is reduced, we believe that the extra appendage material present ventrally in *capu* and *spir* mutant eggs probably results from a change in cell fate, so that follicle cells normally secreting main body chorion are secreting dorsal appendage material instead. A similar reduction in the number of follicle cell imprints in the main body of the eggshell is seen for *fs(1)K10* [Wieschaus 1979; this study (see Table 2)]. The most severe *capu* and *spir* chorion phenotype therefore is similar to that seen in *fs(1)K10*, a maternal-effect mutation that produces a dorsalized chorion and embryo [Wieschaus et al. 1978; Wieschaus 1979, 1980; Wies-

Figure 6. Mutant females contain dorsalized egg chambers. (A and B) Late-stage egg chambers stained with rhodamine-conjugated phalloidin. Anterior is to the left and dorsal is up. (C and D) Egg chambers viewed with differential interference contrast (DIC) microscopy. Anterior is up and dorsal is to the right. Wild-type egg chambers (A and C) exhibit a dorsoventral asymmetry in the shape and position of the follicle cells at the anterior of the oocyte. Follicle cells on the dorsal side are more columnar in this region than those on the ventral side, resulting in greater distance on the dorsal side between the surface of the egg chamber and the oocyte. In some mutant egg chambers [*capu*^{RR}, (B) *capu*^{G7/Df}, (D)], this asymmetry is lost; the distance between the surface of the egg chamber and the oocyte is similar on the dorsal and ventral sides (see arrows). (oc) Oocyte; (fc) follicle cells; (nc) nurse cells.

Figure 7. Mutant females produce eggshells that are dorsialized. In wild-type eggshells, two respiratory appendages extend from the dorsal anterior surface [dorsal appendages (da)]. The follicle cell imprints on the surface of the egg are more elongate on the dorsal side and more regular on the ventral surface. Strongly dorsialized eggs (B and C) have respiratory appendage material secreted around the entire anterior circumference of the egg in a ring and do not exhibit the normal dorsoventral asymmetry in follicle imprint shape. Some mutant eggs have respiratory appendage material in a fused appendage across the dorsal midline (D). Genotypes of the mothers: *fs(1)K10* (B); *spir⁰³* (C and D).

chaus and Szabad 1979). As the dorsal appendage material is also secreted over the dorsal midline in some *capu* and *spir* eggs, the egg phenotype sometimes differs from that of *fs(1)K10*.

The chorion and embryonic phenotypes of *capu* and *spir* are roughly correlated—an egg with more wild-type chorion phenotype usually contains an embryo with weaker abdominal segmentation defects; an egg with the extreme chorion phenotype, if fertilized, usually contains an embryo that has extreme abdominal segmentation defects and is dorsialized (see Table 1).

Table 2. Number of follicle cell imprints counted per egg

Wild type	<i>capu</i>	<i>spir</i>	<i>fs(1)K10</i>
372	RK/Df 265	PJ/PJ 231	312
413	RK/RK 292	03/03 297	296
368	323	313	267
349	289	286	267
388	268	253	291
	292		
Mean 378 ± 24	288 ± 21	276 ± 33	287 ± 20

Follicle cell imprints were counted on two surfaces for every egg, but because of the curvature of the egg, not all follicle cells were counted.

capu and *spir* are required in germ-line-derived cells during oogenesis

To determine whether *capu* and *spir* function in the oocyte–nurse cell complex, genetically mosaic egg chambers were studied. Mosaics in which the oocyte and nurse cells were mutant, but were surrounded by wild-type follicle cells, were constructed by two methods. In the first method, pole cells from homozygous *capu^{RK}* or *spir⁰³* embryos were transplanted into embryos carrying *ovo^{Df}* (see Table 3), a dominant germ-line-dependent female sterile mutation that prevents development of the female germ line (Busson et al. 1983; Perrimon and Gans 1983). In such host females, only the transplanted pole cells can produce eggs. In the second method, germ-line mosaics were induced by irradiation of larvae transheterozygous for *Fs(2)1* (Macdonald and Struhl 1986; Szabad et al. 1987) and either *capu^{RK}* or *spir^{Pf}* according to the dominant female sterile technique (Wieschaus 1980).

For both *capu* and *spir*, the eggs that developed from the mosaic egg chambers exhibited all aspects of the mutant phenotype (data not shown). Examination of live pregastrulation embryos from the mosaic females indicated that the embryos lacked pole cells. Cuticle preparations of unhatched eggs revealed that both dorsialized embryos and embryos with abdominal segmentation defects were produced. In addition, some of the eggs had chorions with fused dorsal appendages. These results indicate that *capu* and *spir* are required in the germ-line-derived oocyte–nurse cell complex. However, the frequency of the more severe phenotypes—dorsialized chorion and embryo—was reduced, which potentially may indicate that the somatic surrounding can modify the expression of the mutant phenotype.

capu and *spir* embryos exhibit defects during cellularization

capu and *spir* mutant females produce a large fraction of embryos that cellularize incorrectly. Many of the blastoderm-stage embryos have patches of cells of different size and density (see Fig. 8). A large percentage of the embryos from females mutant for strong or weak alleles exhibit this aspect of the mutant phenotype (*spir⁴¹*, 57%; *spir^{RP}*, 71%; *capu^{HK3}*, 50%; *capu^{G7/Df}*, 57%). Examination of embryos undergoing cellularization stained with

Table 3. Germ-line mosaics for *capu* and *spir*

Donor parents ^a	No. of injected ♀ that survive to adulthood	No. of mosaic ♀	No. of mosaic ♀ that produce phenotypically wild-type eggs and embryos	No. of mosaic ♀ that produce mutant eggs and embryos
<i>capu</i> /CyO ♀ × <i>capu</i> / <i>capu</i> ♂	24	6	3 (<i>capu</i> /CyO)	3 (<i>capu</i> / <i>capu</i>)
<i>spir</i> /CyO ♀ × <i>spir</i> / <i>spir</i> ♂	25	5	2 (<i>spir</i> /CyO)	3 (<i>spir</i> / <i>spir</i>)

^a Only genotypes with respect to *spir* or *capu* are given. For exact donor genotypes, host genotypes, and test partners, see Materials and methods. Note that the offspring of homozygous females lack pole cells, not the homozygous flies, themselves.

the nucleic acid stain fuchsin (Zalokar and Erk 1976; Wieschaus and Nüsslein-Volhard 1986) indicated that embryos produced by *capu* and *spir* females contain patches of relatively smaller cells that are of a density similar to wild type and patches of larger cells that are less dense than in wild type (see Fig. 8D, E, G, and H). In addition, *spir* mutant females produce embryos in which the larger cells are of a density similar to wild type and the smaller cells are more dense than in wild type (see Table 4). During the early cleavage divisions, embryos containing abnormal numbers of nuclei can be found. In eggs laid by *spir*^{RP} females, we observed 13 with the normal number of nuclei (i.e., 2ⁿ), but 5 with abnormal numbers (5, 5, 14, 14, 23). From *capu*^{G7/Df} females, 12 embryos with the normal number of nuclei were counted and 2 with abnormal numbers (24, 26). In addition, the nuclei often fail to migrate properly along the anteroposterior axis, leading to abnormal distributions of nuclei when they arrive at the periphery. Time-lapse video films of embryos from *capu*^{RR} and *spir*^{RP} mutant females indicate that the stages of nuclear division immediately preceding cellularization often are asynchronous.

Epistatic relationships of the loci that influence patterning of the egg and embryo

Because mutations in *capu* and *spir* cause absence of pole cells, loss of abdominal segmentation, and variable dorsalization of eggshell and embryo, it seemed possible that the *capu*,*spir* double-mutant phenotype would be more extreme and cause all eggs and embryos to be dorsalized. The phenotype of offspring from females homozygous for the strong alleles *capu*^{RR},*spir*⁰³ is, however, similar to that of either mutant by itself (data not shown). In particular, the frequency of the dorsalized phenotype does not increase significantly in the double mutant.

To establish the epistatic relationship between *capu* and *spir* and the other genes that affect patterning of the eggshell and embryo, eggs laid by females doubly mutant for all of the pairwise combinations of *capu* or *spir* with *fs(1)K10*, *grk*, and *top* were examined. *fs(1)K10* is a recessive female sterile locus in which both the eggshell and embryo are dorsalized (Wieschaus et al. 1978; Wieschaus 1979, 1980; Wieschaus and Szabad 1979). Both *fs(1)K10*,*spir*⁰³ and *fs(1)K10*,*capu*^{RR} double-mutant females produce eggs with strongly dorsalized chorions, similar to those produced by *fs(1)K10* females. Eggs from *fs(1)K10*,*spir*⁰³ are not fertilized, presumably because the

strong dorsalization of the chorion prevents sperm entry (through the micropyle). Approximately 1% of the eggs produced by *fs(1)K10* females are fertilized, suggesting that the chorion phenotype of eggs from *fs(1)K10*,*spir*⁰³ females is somewhat enhanced. The embryos produced by *fs(1)K10*,*capu*^{RR} females always are dorsalized also. Observation of pregastrulation embryos indicates that they lack pole cells. *grk* and *top* are recessive female sterile loci that produce ventralized eggs and embryos (Schüpbach 1987). These eggs are elongated, with fused and reduced dorsal appendages. The embryonic cuticle consists only of a patch of ventral cuticle. In *capu*^{RR} *grk*^{WG}, in *grk*^{WG} *spir*^{RP}, in *capu*^{RR} *top*¹, and in *spir*^{RR} *top*¹, both the egg and embryo are ventralized, indicating that the ventralizing loci *grk* and *top* are epistatic to the dorsalizing action of *capu* and *spir* in the dorsoventral axis. The ventralized embryos from the double-mutant females also have abdominal segmentation defects and lack pole cells, indicating that the abdominal segmentation defects of *capu* and *spir* are additive to and independent of the dorsoventral defects.

Discussion

Females homozygous for *capu* and *spir* produce eggs and embryos with very specific pattern defects. All of the embryos lack pole cells and most have abdominal segmentation defects. These defects in the anteroposterior embryonic pattern are similar to those produced by the *grandchildless-knirps* group of maternal-effect loci. Some alleles also produce eggs and embryos that are dorsalized, similar to those produced by the dorsalizing group of maternal-effect loci and *fs(1)K10*.

The different pattern phenotypes observed correlate with the amount of gene activity. Females homozygous for certain alleles produce mostly embryos with pole cell and segmentation defects. These alleles are presumably homomorphic, because the phenotype is more severe when the allele is transheterozygous with a deficiency for the locus. Although we are uncertain whether any of the alleles represent complete loss of gene function, those alleles that produce a high frequency of severe phenotypes, such as eggs and embryos that are dorsalized, are probably closer to amorphic, because the phenotype produced is not altered significantly when transheterozygous with a deficiency for the locus. Therefore, the allelic series indicates that pole cell formation has the highest requirement for functional gene product, with abdominal segmentation and dorsalization requiring progressively less.

Figure 8. Embryos from mutant females exhibit abnormal nuclear densities and asynchronous cellularization during the syncytial blastoderm stage. (A–E) Embryos stained with fuchsin. (A–C) Wild type. B and C show progressive stages of cellularization in different wild-type embryos. (D and E) An embryo exhibiting two different stages of cellularization. The higher magnification view in D was taken from the region between the two arrows in E. The region to the left of the arrow in D is more similar in stage to the wild type shown in B, whereas the region to the right of the arrow in D is more similar in stage to the wild type shown in C. The region shown to the right of the arrow in D is similar in nuclear density to wild type. (F and G) Surface views of wild-type (F) and mutant (G) embryos stained with rhodamine-conjugated phalloidin to visualize the cell membranes. (H) Hoechst staining of the same region of the mutant embryo shown in G. Cells and nuclei to the right of the arrow in G and H are more comparable in size and density to wild type. Those cells and nuclei to the left of the arrow are less dense and larger than wild type. Genotypes of mothers: *spir*^{RP} (D and E); *spir*^{RP}/*Df* (G and H).

Within an individual egg, the chorion and embryonic phenotypes are roughly correlated—an egg with a more wild-type chorion phenotype usually contains an embryo with weaker segmentation defects, and an egg with the extreme dorsalized chorion phenotype, if fertilized, usually contains an embryo that has extreme abdominal segment deletions and is also dorsalized. Because this correlation is not absolute, the embryonic pattern probably does not result directly from the chorion pattern or from the pattern of follicle cells during oogenesis. Rather, both the embryonic and the follicle cell patterns reflect the results of a signaling process between the soma and the germ line (see below). Although both pat-

terns probably result from the same signaling events, the final patterns are many steps removed from the actual signaling process, allowing for differences within an individual egg in the final phenotypes.

Given that there are several mutations that affect the dorsoventral patterning of oogenesis and embryogenesis simultaneously, it seems that dorsoventral patterning of the eggshell and embryo are linked. Mutations in *grk* and *top* (Schüpbach 1987) cause ventralization during oogenesis and embryogenesis, whereas mutations in *capu*, *spir*, and *fs(1)K10* cause dorsalization of both processes. At present, it is unknown whether a direct link exists between the anteroposterior pattern of the egg-

Table 4. Nuclear density in cellularizing embryos

Neighboring patches of the same embryo									
wild type	capu			spir ^a					
				a		b		c	
71	65	43	71	46	87	40	59	103	
51	76	49	61	37	87	56	68	124	
70	57	35	62	49	92	51	64	142	
59	71	45	62	30					
79	73	37	67	37					
84	70	47	55	37					
55									
Mean	67 ± 12	69	43	63	40	89	49	64	123
Ratio	1.6			1.6		1.8		0.5	

Genotypes used were *capu*^{G7/Df} and *spir*^{RP/spir}^{RP}. Density is given as number of nuclei per 1338.5 mm².

^a *spir* embryos are divided into categories: (a) those that have regions less dense; (b) of intermediate density; and (c) more dense than wild type.

shell and embryo. There are no good markers for the spatial pattern of the main body of the eggshell that would allow the detection of such a link. However, the posterior pole of the eggshell is marked by a specialized structure called the aeropyle. Although the posterior pole of the oocyte is defective in *capu* and *spir*, the aeropyle is present on the eggshells.

The relationship between the cellularization defects and the anteroposterior and dorsoventral pattern alterations in *capu* and *spir* mutant embryos is not clear. The *grandchildless* phenotype is apparent in the defective polar plasm of the mutant embryos before any observable nuclear density defects. This argues that the *grandchildless* phenotype does not result from these defects. In addition, Sullivan (1987) demonstrated that anteroposterior patterning, as visualized by *ftz* expression, occurred independent of cellular density. This indicates that the abdominal segment deletions seen in *capu* and *spir* embryos do not result from abnormal cell densities. In the dorsoventral axis, the alterations in the expression of both *zen* and *twist* are similar to those produced by the other dorsalizing mutations. It is therefore possible that *capu* and *spir* alter the function of one of the dorsal group genes and that the cellularization defects and dorsalization are also independent of one another. Defects similar to those seen in *capu* and *spir* in the density of nuclei and cells at the time of cellularization were observed in gynandromorphs by Zussman and Wieschaus (1987). These gynandromorphs survive to adulthood, without the pattern alterations seen in *capu* and *spir*, providing an additional argument that having different cell densities at the cellular blastoderm stage is not sufficient to cause the pattern alterations present in *capu* and *spir* embryos. It is interesting to note that Zalkov and Erk (1976) produced embryos with similar nuclear migration and cellularization defects by treatment with cytochalasin B, a drug that inhibits polymerization of actin. This suggests that *capu* and *spir* might be affecting an actin-based transport system in the early embryo.

capu and *spir* function in signaling processes in the germ line

Germ-line mosaics of *capu* and *spir*, made either by pole cell transplantation or mitotic recombination in the germ line, indicate that *capu* and *spir* are required in the nurse cell–oocyte complex. Because mutations in *capu* or *spir* in the germ-line-derived nurse cell–oocyte complex cause an abnormal behavior of the somatically derived follicle cells, lack of *capu* or *spir* activity must be somehow disrupting the signaling events between the oocyte and follicle cells that are required for the normal dorsoventral pattern of the egg chamber and embryo. Similar results have been obtained for *fs(1)K10* (Wieschaus et al. 1978) and *grk* (Schüpbach 1987). Whether these gene products function directly in the intercellular communication process or are involved in establishing the underlying pattern of the oocyte (which is then conveyed to the follicle cells) is uncertain. However, because *capu* and *spir* seem to be required for a number of patterns in the oocyte, we think it more likely that they are required for establishing or maintaining the dorsoventral pattern of the germ cell and are not directly involved in the production of the intercellular signal.

top, on the other hand, is a gene that functions in the somatically derived follicle cells. *top* also is involved in a signaling process because follicle cells that are mutant for *top* results in ventralization of the embryo (Schüpbach 1987). The recent finding that *top* encodes the *Drosophila* homolog of the epidermal growth factor (EGF) receptor suggests that *top* might play a more direct role in the intercellular signaling process (Price et al. 1989; Schejter and Shilo 1989).

The epistatic relationships between the genes involved in the signaling process suggested an ordering of the genes in the early steps in dorsoventral patterning (see Fig. 9). We placed the ventralizing loci *grk* and *top* downstream of the dorsalizing loci *capu*, *spir*, and *fs(1)K10*, because the ventralizing loci are epistatic to the dorsalizing loci. Because the dorsalizing action of mutations in *capu*, *spir*, and *fs(1)K10* can be observed only in the presence of the normal *grk* and *top* gene products, we believe that *grk* and *top* mediate the effects of *capu*, *spir*, and *fs(1)K10*. For instance, *grk* and *top* could be producing an intercellular signal in response to the underlying asymmetries produced by genes like *capu*, *spir*, and *fs(1)K10*. In the absence of *grk* and *top* gene function, then, the effects of *capu*, *spir*, and *fs(1)K10* would be lost.

Possible roles for *capu* and *spir*

capu and *spir* mutations disrupt formation or stability of polar granules. This suggests that the mutant egg chambers are defective in the production of components of the polar granules, in localization of the components to the posterior end of the egg chamber, in stability of the polar granules, or in organization of the posterior cytoplasm in general.

The double mutants of *Bic-D* with *capu* and *spir* indicate that *capu* and *spir* are not required for abdominal

Figure 9. A working model for the early steps in dorsoventral patterning. This model combines the analysis of the mutant phenotypes, the epistatic relationships between the genes, the cell type in which the gene functions, and molecular information about the location and nature of the *top* and *fs(1)K10* gene products. Within the egg chamber there must be an initial dorsoventral asymmetry, the nature of which is unknown. On the ventral side of the oocyte, this asymmetry results in low activity levels of a signaling process between the somatically derived follicle cells and the germ-line-derived oocyte–nurse cell complex. In the absence of *fs(1)K10*, *capu*, or *spir* (blocking the pathway at A), the signaling process occurs at high levels on the ventral side of the egg chamber, resulting in dorsalization of the egg and embryo. Because of its location within the oocyte nucleus and the presence of a DNA recognition helix (Proust et al. 1988), the *fs(1)K10* gene product is a good candidate for negatively regulating expression of the germ-line signal, either directly or through the action of intermediate genes. We suggest that *capu* and *spir* might be involved in establishing or maintaining the proper spatial distribution of the germ-line signal [again either directly or indirectly] by analogy to their proposed role in antero-posterior patterning (see Discussion). Because *top* functions in the soma (Schüpbach 1987) and encodes the *Drosophila* homolog of the EGF receptor (Price et al. 1989; Schejter and Shilo 1989), it is thought to have a direct role in the intercellular signaling process, probably as the somatic receptor for the germ-line signal. Both *grk* and *top* mutant females produce ventralized eggs and embryos (block at B). Because *grk* functions in the germ line and directs behavior of the follicle cells (Schüpbach 1987), it is probably directly or indirectly involved in the production of the germ-line signal. The high levels of the intercellular signaling activity on the dorsal side of the egg chamber result in dorsal-type follicle cell determination and in the ultimate repression of production of the ventral morphogen on the dorsal side of the egg. Because *top* acts in the soma but regulates ventral morphogen production in the egg, activation of *top* has to be signaled back to the germ line. There are additional members of the dorsal group of maternal-effect genes that act in the soma (T. Schüpbach, unpubl.; C. Nüsslein-Volhard, pers. comm.). The function of these genes is unknown, but they might be involved in modification of the somatic signal and/or transmission of the somatic signal back to the germ line. The repressing effect on ventral morphogen production by *top* on the dorsal side then occurs before the action of these genes, before the signal is transmitted back to the germ line. On the ventral side of the egg chamber, the low level of the intercellular signaling process results in ventral-type follicle cell determination and high levels of ventral morphogen. The germ line-dependent genes of the *dorsal*–*Toll* group are responsible for the production of ventral morphogen. Blocking the pathway at C results in the production of dorsalized embryos in wild-type eggshells. For the purposes of simplification, the model shows extremes of expression that probably exist only at the dorsal-most and ventral-most regions of the dorsoventral axis. We expect that, in reality, the levels of activity are graded along the axis.

segmentation in the anterior half of a bicaudal embryo. Because normal abdominal segments form in the double mutant, *capu* and *spir* do not seem to be involved primarily in the production of the abdominal segmentation determinant. It has been shown that the posterior cytoplasm of a *Drosophila* egg contains the determinant for abdominal segmentation, as well as the polar granules (Frohnhofer 1986; Lehmann and Nüsslein-Volhard 1986). *Bic-D* most likely misplaces posterior determinants to the anterior end of the egg (Frohnhofer 1986; Lehmann and Nüsslein-Volhard 1986). In the *Bic-D* double mutants, *Bic-D* is probably still causing misloca-

tion of the abdominal segmentation determinant to the anterior pole, thus circumventing the requirement for *capu* and *spir* in abdominal segmentation at the anterior end of the bicaudal embryo. This line of reasoning suggests two alternative models for how normal abdominal segmentation occurs in the anterior half of the bicaudal embryo. In the first model, the *capu* and *spir* gene products normally might be involved in transporting the abdominal segmentation determinant (and possibly also components of the polar granules) to (or stabilizing it at) the posterior pole. By causing localization and/or stabilization of the posterior determinant in the anterior of the

oocyte, *Bic-D* directly circumvents the requirement for the *capu* and *spir* gene products, resulting in normal abdominal segmentation in the anterior half of the egg. In an alternate model, *capu* and *spir* gene products normally might be involved in activation or utilization of the posterior determinant, but the anterior half of the egg contains gene products that can substitute for this function, resulting in normal abdominal segmentation in the anterior of a bicaudal egg. However, because the *vasa* antibody staining indicates that *vasa* protein is not concentrated in the posterior plasma in *capu* and *spir* oocytes, we believe the first model is more reasonable—that *capu* and *spir* gene products are required for localization or stabilization of substances at the posterior pole of the egg chamber.

If *capu* and *spir* are involved directly in localization or stabilization of substances to the posterior pole of the oocyte for anteroposterior patterning, it would seem most reasonable to propose that they act similarly in dorsoventral patterning. There are no visible, asymmetrically localized structures in the oocyte or embryo that are thought to be involved in specifying pattern along the dorsoventral axis. However, the *Drosophila* embryo does not develop randomly in the egg but always has its dorsoventral pattern aligned with that of the eggshell. Therefore, the embryonic dorsoventral axis is also already predetermined in oogenesis, and some factor or structure must be asymmetrically distributed dorsoventrally. The earliest visible dorsoventral asymmetry in the oocyte during oogenesis is the asymmetric localization of the oocyte nucleus. Parks and Spradling (1987) observed dorsoventral asymmetries in expression of chorion genes during stage 10B of oogenesis. Follicle cells situated over the oocyte nucleus in the dorsal anterior region of the oocyte express certain chorion genes earlier than other follicle cells. These investigators suggest that given the proximity of these follicle cells to the oocyte nucleus, a signal from the oocyte nucleus to the follicle cells may be responsible for establishing this asymmetric chorion gene expression. As discussed above, a similar asymmetric signal from the oocyte might establish the dorsoventral pattern of the eggshell and embryo, possibly through the action of *grk* and *top*. Therefore, *capu* and *spir* might be involved in the localization or stabilization of asymmetric substances that are necessary for the production of any of these dorsoventral asymmetries.

According to this model, the phenotypic variability observed for *capu* and *spir* could be explained as different sensitivities in the dorsoventral and anteroposterior axes to loss of a transport system. Because even the weakest alleles cause failure of polar granule formation and some abdominal segmentation defects, the anteroposterior axis would have to be considered more sensitive to loss of the asymmetric distribution of substances. Because dorsoventral pattern defects become frequent only in the stronger alleles, the dorsoventral axis may be less sensitive either because the distances involved are shorter or because substances do not have to be as tightly localized for proper function. This also

might explain the variation in dorsalization seen in the mutants.

It is possible that *capu* and *spir* might function by simultaneously regulating members of the *grandchildless-knirps* and dorsalizing groups of loci. Because *staufen* (Schüpbach and Wieschaus 1986) is the only identified member of the *grandchildless-knirps* group of loci to produce the same phenotype as *capu* and *spir* in combination with *Bic-D* (R. Lehmann, pers. comm.), *staufen* is the only candidate within this class of loci that could be regulated directly by *capu* and *spir*. It seems unlikely that *capu* and *spir* are regulating *staufen*, however, because *capu* and *spir* mutant embryos do not have the head defects typical of *staufen* mutant embryos. The possibility exists that there are additional, unidentified loci with the *grandchildless-knirps* mutant phenotype that would be better candidates for being regulated directly by *capu* and *spir*. Because of the similarities between the severely dorsalized *capu* and *spir* chorion and embryo phenotypes and the *fs(1)K10* phenotype, it is tempting to speculate that *capu* and *spir* might be regulating *fs(1)K10* in the dorsoventral axis. The *fs(1)K10* mutant phenotype, however, is not variable like that of *capu* and *spir*. This model would therefore suggest that mutations in *capu* and *spir* are not completely eliminating *fs(1)K10* function but, rather, decreasing expression or function of *fs(1)K10*.

Materials and methods

Genetics

Three alleles of *capu* and four of *spir* were isolated in a screen for female steriles on the second chromosome (Schüpbach and Wieschaus 1989). Six alleles of *spir* were isolated fortuitously in a similar screen for female sterile mutations on the third chromosome in the laboratory of C. Nüsslein-Volhard. Two alleles of *spir* and one of *capu* were isolated by R. Clifford in a screen for mutations causing lethality or female sterility in combination with a chromosome carrying *spir^{RP}* and *capu^{RK}*. All alleles were induced with ethylmethane sulfonate (EMS), except for *capu^{G7}*, which was induced with γ -rays.

The map position of *capu^{RK}* was found to be 2–8, whereas that of *spir^{RP}* was found to be 2–54.5. The deficiency used to uncover *capu*, *Df(2L)ed^{SZ1.2}* (Reuter and Szidonya 1983; Semeshin and Szidonya 1985), is deleted for the region between 24A3-4 and 24D3-4, whereas those used to uncover *spir* are *Df(2L)SD-72^{pr26}*, which is deficient from 37D5-6 to 38C8-10, and *Df(2L)SD-72^{pr21}*, which is deficient from 37E3-F1 to 38C6-10 (Brittner and Ganetzky 1983).

Characterization of mutant phenotype

For microscopic examination of chorions and embryonic cuticular phenotypes, eggs and embryos were treated as in Wieschaus and Nüsslein-Volhard (1986). Embryos were prepared for sectioning as in Schüpbach and Wieschaus (1986). Egg chambers were stained with rhodamine-conjugated phalloidin or prepared for DIC as described for embryos in Wieschaus and Nüsslein-Volhard (1986). Follicle cell imprints and nuclear densities were counted using a Mac II and Image 1.03 image analysis software designed by Wayne Rasband. Eggs with the most severe dorsalized phenotype were selected from *spir* and *capu* and compared to eggs from wild type. All visible imprints

on both surfaces were counted, but because of the spherical nature of the egg, it is not possible to count follicle cell imprints on the edges of the surface. Nuclear densities were counted in embryos stained with fuchsin, as described in Wieschaus and Nüsslein-Volhard (1986). Defects during cellularization have been observed in embryos from *capu^{HK3}/capu^{HK3} capu^{RR}/capu^{RR}*, *capu^{RR} Df(2L)ed^{SZ1.2}*, *capu^{G7}/Df(2L)ed^{SZ1.2}*, *spir^{RP}/spir^{RP}*, and *spir^{RP}/Df(2L)pr²¹*, but not from *spir^{RP}/CyO spir⁴¹/spir⁴¹* or *capu^{G7}/CyO*.

Immunocytochemistry

Ovaries were prepared for cryostat sectioning as described for embryos in Hafen et al. (1983), except that 0.1 M phosphate buffer (pH 7.6) was used instead of phosphate-buffered saline (PBS). Sections were 12- to 15- μ m thick. Subsequent treatment of the sections and immunostaining of the ovary sections with the polar granule antibody (mAb 46F11) was conducted as in Hay et al. (1988b), using a 1 : 500 dilution of affinity-purified antibody (kindly provided by B. Hay). Embryos were stained with this antibody, as described in Hay et al. (1988b). Embryos for *zen* and β -galactosidase antibody staining were dechorionated in bleach, fixed in a mixture of 9 : 1 heptane/3.7% formaldehyde in PBS for 5 min, and devitellinized using the modification of the Mitchison and Sedat (1983) procedure described in Wieschaus and Nüsslein-Volhard (1986). After rehydration through a series of solutions containing decreasing concentrations of alcohol, the embryos were incubated overnight at 4°C with the antibody. The *zen* antibody (kindly provided by Chris Rushlow) was diluted 1 : 20, the antibody against β -galactosidase (Promega) was diluted 1 : 1000, and the polar granule antibody (kindly provided by B. Hay) was diluted 1 : 5. Antibody localization was visualized using a Vectastain kit (Vectastain peroxidase ABC kit, Vector Labs)—either with biotinylated goat anti-mouse (β -galactosidase, polar granules) or biotinylated goat anti-rabbit (*zen*) avidin—horseradish peroxidase-complexed antibody. Stained embryos were then dehydrated through an increasing methanol series and mounted on glass slides in methyl salicylate for observation.

Germ-line mosaics

Pole cell transplants were conducted as described in Van Deusen (1976). Crosses used to generate pole cell donors for *capu* were *capu^{RR} cn bw/CyO* ♀ × *capu^{RR} cn bw /capu^{RR} cn bw* ♂, and for *spir* were *spir⁰³/CyO* ♀ × *spir⁰³/spir⁰³* ♂. The cross used to generate recipients was *y v f mal/y v f mal* ♀ × *ovo^{D1} v mal/Y* ♂. The recipient females carry *ovo^{D1}*, a dominant germ-line-dependent female sterile mutation that prevents development of the female germ line (Busson et al. 1983; Perrimon and Gans 1983; Oliver et al. 1987). Thus, only successfully transplanted pole cells will produce eggs. Adult recipient females were mated with the following test partners: for *capu*, *capu^{RR} cn bw/capu^{RR} cn bw* ♂; for *spir*, *spir⁰³/spir⁰³* ♂.

Germ-line mosaics were also induced by irradiation of larvae transheterozygous for *Fs(2)1* (Macdonald and Struhl 1986) and either *capu^{RR}* or *spir^{PI}*, as in the dominant female sterile technique (Wieschaus 1980). Larvae that were 40 ± 6 hr were irradiated with ~1300 γ -rays. Females carrying *Fs(2)1* produce very small, abnormal eggs. Females carrying clones of germ-line cells that had lost *Fs(2)1* (and thus, are homozygous for *capu* or *spir^l*) lay normal-size eggs.

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References

- Akam, M. 1987. The molecular basis for metameric pattern in the *Drosophila* embryo. *Development* **101**: 1–22.
- Anderson, K.V. 1987. Dorsal-ventral embryonic pattern genes of *Drosophila*. *Trends Genet.* **3**(4): 91–97.
- Boswell, R.E. and A.P. Mahowald. 1985. *tudor*, a gene required for assembly of the germ plasm in *Drosophila melanogaster*. *Cell* **43**: 97–104.
- Brittnacher, J. and B. Ganetzky. 1983. On the components of segregation distortion in *Drosophila melanogaster*. II. Deletion mapping and dosage analysis of the SD locus. *Genetics* **103**: 659–673.
- Busson, D., M. Gans, K. Komitopoulou, and M. Masson. 1983. Genetic analysis of three dominant female sterile mutations located on the X-chromosome of *Drosophila melanogaster*. *Genetics* **105**: 309–325.
- Carroll, S.B. and M.P. Scott. 1985. Localization of the *fushi tarazu* protein during *Drosophila* embryogenesis. *Cell* **43**: 47–57.
- Carroll, S., G. Winslow, T. Schüpbach, and M. Scott. 1986. Maternal control of *Drosophila* segmentation gene expression. *Nature* **323**: 278–280.
- Carroll, S., G. Winslow, V. Twombly, and M. Scott. 1987. Genes that control dorsoventral polarity affect gene expression along the anteroposterior axis of the *Drosophila* embryo. *Development* **99**: 327–332.
- Degelmann, A., P.A. Hardy, N. Perrimon, and A.P. Mahowald. 1986. Developmental analysis of the *torso*-like phenotype in *Drosophila* produced by a maternal-effect locus. *Dev. Biol.* **115**: 479–489.
- Doyle, H.J., K. Harding, T. Hoey, and M. Levine. 1986. Transcripts encoded by a homeobox gene are restricted to dorsal tissues of *Drosophila* embryos. *Nature* **323**: 76–79.
- Driever, W. and C. Nüsslein-Volhard. 1988a. A gradient of *bicoid* protein in *Drosophila* embryos. *Cell* **54**: 83–93.
- . 1988b. The *bicoid* protein determines position in the *Drosophila* embryo in a concentration-dependent manner. *Cell* **54**: 95–104.
- Frohnhofer, H.G. and C. Nüsslein-Volhard. 1986. Organization of anterior pattern in the *Drosophila* embryo by the maternal gene *bicoid*. *Nature* **324**: 120–125.
- . 1987. Maternal genes required for the anterior localization of *bicoid* activity in the embryo of *Drosophila*. *Genes Dev.* **1**: 880–890.

- Frohnhofer, H.G., R. Lehmann, and C. Nüsslein-Volhard. 1986. Manipulating the anteroposterior pattern of the *Drosophila* embryo. *J. Embryol. Exp. Morphol.* **97S**: 169–179.
- Hafen, E., A. Kuriowa, and W. Gehring. 1984. Spatial distribution of transcripts from the segmentation gene *fushi tarazu* during *Drosophila* embryonic development. *Cell* **37**: 833–841.
- Hafen, E., M. Levine, R.L. Garber, and W.J. Gehring. 1983. An improved *in situ* hybridization method for the detection of cellular RNAs in *Drosophila* tissue sections and its application for localizing transcripts of the homeotic *Antennapedia* gene complex. *EMBO J.* **2**(4): 617–623.
- Hay, B., L.Y. Jan, and Y.M. Jan. 1988a. A protein component of *Drosophila* polar granules is encoded by *vasa* and has extensive sequence similarity to ATP-dependent helicases. *Cell* **55**: 577–587.
- Hay, B., L. Ackerman, S. Barbel, L. Jan, and Y. Jan. 1988b. Identification of a component of *Drosophila* polar granules. *Development* **103**: 625–640.
- Hiromi, Y., A. Kuriowa, and W. Gehring. 1985. Control elements of the *Drosophila* segmentation gene *fushi tarazu*. *Cell* **43**: 603–613.
- Ingham, P.W. 1988. The molecular genetics of embryonic pattern formation in *Drosophila*. *Nature* **335**: 25–34.
- King, R.C. 1970. Ovarian development in *Drosophila melanogaster*. Academic Press, New York.
- Klingler, M., M. Erdelyi, J. Szabae, and C. Nüsslein-Volhard. 1988. Function of *torso* in determining the terminal anlagen of the *Drosophila* embryo. *Nature* **335**: 275–277.
- Lehmann, R. 1985. 'Regionsspezifische segmentierungsmutanten bei *Drosophila melanogaster* meigen.' PhD. thesis. Eberhard-Karls-Universität, Tübingen.
- . 1988. Phenotypic comparison between maternal and zygotic genes controlling the segmental pattern of the *Drosophila* embryo. *Development* **104S**: 17–27.
- Lehmann, R. and C. Nüsslein-Volhard. 1986. Abdominal segmentation, pole cell formation, and embryonic polarity require the localized activity of *oskar*, a maternal gene in *Drosophila*. *Cell* **47**: 141–152.
- . 1987. Involvement of the *pumilio* gene in the transport of an abdominal signal in the *Drosophila* embryo. *Nature* **329**: 167–170.
- Levine, M. 1988. Molecular analysis of dorsal-ventral polarity in *Drosophila*. *Cell* **52**: 785–786.
- Macdonald, P.M. and G. Struhl. 1986. A molecular gradient in early *Drosophila* embryos and its role in specifying the body pattern. *Nature* **324**: 537–545.
- Mahowald, A.P., C.D. Allis, K.M. Karrer, E.M. Underwood, and G.L. Waring. 1979. Germ plasm and pole cells of *Drosophila*. In *Determinants of spatial organization*. (ed. S. Subtelney and I.R. Koenigsberg) p. 127–146. Academic Press, New York.
- Mitchison, T.J. and J. Sedat. 1983. Localization of antigenic determinants in whole *Drosophila* embryos. *Dev. Biol.* **99**: 261–264.
- Mohler, J. and E.F. Wieschaus. 1985. Bicaudal mutations of *Drosophila melanogaster*: Alteration of blastoderm cell fate. *Cold Spring Harbor Symp. Quant. Biol.* **50**: 105–111.
- . 1986. Dominant maternal-effect mutations of *Drosophila melanogaster* causing the production of double-abdomen embryos. *Genetics* **112**: 803–822.
- Nüsslein-Volhard, C. 1979. Maternal effect mutations that alter the spatial coordinates of the embryo of *Drosophila melanogaster*. In *Determinants of spatial organization*. (ed. S. Subtelney and I.R. Koenigsberg), pp. 185–211. Academic Press, New York.
- Nüsslein-Volhard, C., H.G. Frohnhofer, and R. Lehmann. 1987. Determination of anteroposterior polarity in *Drosophila*. *Science* **238**: 1675–1681.
- Oliver, B., N. Perrimon, and A.P. Mahowald. 1987. The *ovo* locus is required for sex-specific germ line maintenance in *Drosophila*. *Genes Dev.* **1**: 913–923.
- Parks, S. and A. Spradling. 1987. Spatially regulated expression of chorion genes during *Drosophila* oogenesis. *Genes Dev.* **1**: 497–509.
- Perrimon, N. and N. Gans. 1983. Clonal analysis of the tissue specificity of recessive female-sterile mutation *Fs(1)K1237*. *Dev. Biol.* **105**: 404–414.
- Price, J.V., R.J. Clifford, and T. Schüpbach. 1989. The maternal ventralizing locus *torpedo* is allelic to *faint little ball*, an embryonic lethal, and encodes the *Drosophila* EGF receptor homolog. *Cell* **56**: 1085–1092.
- Proust, E., F. Deryckere, C. Roos, M. Haenlin, V. Pantescio, and E. Mohier. 1988. Role of the oocyte nucleus in determination of the dorsoventral polarity of *Drosophila* as revealed by molecular analysis of the *K10* gene. *Genes Dev.* **2**: 891–900.
- Reuter, G. and J. Szidonya. 1983. Cytogenetic analysis of variegation suppressors and dominant temperature-sensitive lethal in region 23-26 of chromosome 2L in *Drosophila melanogaster*. *Chromosoma* **88**: 277–285.
- Rushlow, C., M. Frasch, H. Doyle, and M. Levine. 1987. Maternal regulation of *zerknüllt*: A homeobox gene controlling differentiation of dorsal tissues in *Drosophila*. *Nature* **330**: 583–586.
- St. Johnston, R.D. and W.M. Gelbart. 1987. Decapentaplegic transcripts are localized along the dorsal-ventral axis of the *Drosophila* embryo. *EMBO J.* **6**(9): 2785–2791.
- Schejter, E.D. and B. Shilo. 1989. The *Drosophila* EGF receptor homolog (DER) gene is allelic to *faint little ball*, a locus essential for embryonic development. *Cell* **56**: 1093–1114.
- Schüpbach, T. 1987. Germline and soma cooperate during oogenesis to establish the dorso-ventral pattern of egg shell and embryo in *Drosophila melanogaster*. *Cell* **49**: 699–707.
- Schüpbach, T. and E. Wieschaus. 1986. Maternal-effect mutations altering the anterior-posterior pattern of the *Drosophila* embryo. *Wilhelm Roux's Arch. Dev. Biol.* **195**: 302–317.
- . 1989. Female sterile mutations on the second chromosome of *Drosophila melanogaster*. I. Maternal effect mutations. *Genetics* **121**: 101–117.
- Scott, M. and P.H. O'Farrell. 1986. Spatial programming of gene expression in early *Drosophila* embryogenesis. *Annu. Rev. Cell Biol.* **2**: 49–80.
- Semeshin, V.F. and J. Szidonya. 1985. EM mapping of rearrangements in the 24-25 sections of *D. melanogaster* 2L chromosomes. *Dros. Inf. Serv.* **61**: 148–154.
- Steward, R., S.B. Zusman, L.H. Huang, and P. Schedl. 1988. The *dorsal* protein is distributed in a gradient in early *Drosophila* embryos. *Cell* **55**: 487–495.
- Strecker, T.R., S.R. Halsell, W.W. Fisher, and H.D. Lipschitz. 1989. Reciprocal effects of hyper- and hypoactivity mutations in the *Drosophila* pattern gene *torso*. *Science* **243**: 1062–1066.
- Sullivan, W. 1987. Independence of *fushi tarazu* expression with respect to cellular density in *Drosophila* embryos. *Nature* **327**: 164–167.
- Szabad, J., M. Erdelyi, and J. Szidonya. 1987. Characterization of *FS(2)1*, a germ-line dependent dominant female sterile mutation of *Drosophila*. *Acta Biol. Hun.* **38**(2): 257–266.
- Thisse, B., C. Stoetzel, C. Gorostiza-Thisse, and F. Perrin-Schmitt. 1988. Sequence of the *twist* gene and nuclear local-

- ization of its protein in endomesodermal cells of early *Drosophila* embryos. *EMBO J.* **7**(7): 2175–2183.
- Van Deusen, E.B. 1976. Sex determination in germ line chimeras of *Drosophila melanogaster*. *J. Embryol. Exp. Morphol.* **37**: 173–185.
- Wieschaus, E. 1979. *fs(1)K10*, a female sterile mutation altering the pattern of both the egg coverings and the resultant embryos in *Drosophila*. In *Cell lineage, stem cell and cell differentiation* (ed. N. LeDouarin), pp. 291–302, Elsevier/North-Holland Biomedical Press, New York.
- . A combined genetic and mosaic approach to the study of oogenesis in *Drosophila*. Development and neurobiology of *Drosophila*, (ed. O. Siddiqi, P. Babu, L.M. Hall, and J.C. Hall), pp. 85–94, Plenum Publishing, New York.
- Wieschaus, E. and C. Nüsslein-Volhard. 1986. Looking at embryos. *Drosophila: A practical approach*. (ed. D.B. Roberts), pp. 199–228, IRL Press, Oxford, Washington D.C.
- Wieschaus, E. and J. Szabad. 1979. the development and function of the female germ line in *Drosophila melanogaster*: A cell lineage study. *Dev. Biol.* **68**: 29–46.
- Wieschaus, E., J.L. Marsh, and W. Gehring. 1978. *fs(1)K10*, a germline-dependent female sterile mutation causing abnormal chorion morphology in *Drosophila melanogaster*. *Wilhelm Roux's Arch. Dev. Biol.* **184**: 75–82.
- Zalokar, M. and I. Erk. 1976. Division and migration of nuclei during early embryogenesis of *Drosophila melanogaster*. *J. Microsc. Biol. Cell* **25**: 97–106.
- Zusman, S.B. and E. Wieschaus. 1987. A cell marker system and mosaic patterns during early embryonic development in *Drosophila melanogaster*. *Genetics* **115**: 725–736.

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