The relationship between ovarian and embryonic dorsoventral patterning in *Drosophila*

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**SUMMARY**

In *Drosophila*, the dorsoventral asymmetry of the egg chamber depends on a dorsalizing signal that emanates from the oocyte. This signal is supplied by the TGF-α-like *gurken* protein whose RNA is localized to the dorsal-anterior corner of the oocyte. *gurken* protein is the potential ligand of the *Drosophila* EGF receptor homolog (torpedo), which is expressed in the follicular epithelium surrounding the oocyte. Here, we describe how changes in the dorsalizing germ-line signal affect the embryonic dorsoventral pattern. A reduction in strength of the germ-line signal as produced by mutations in *gurken* or *torpedo* does not change the slope of the embryonic dorsoventral morphogen gradient, but causes a splitting of the gradient ventrally. This leads to embryos with two partial dorsoventral axes. A change in distribution of the germ-line signal as caused by *fs(1)K10, squid* and *orb* mutations leads to a shift in the orientation of the embryonic dorsoventral axis relative to the anterior-posterior axis. In extreme cases, this results in embryos with a dorsoventral axis almost parallel to the anterior-posterior axis. These results imply that *gurken*, unlike other localized cytoplasmic determinants, is not directly responsible for the establishment of cell fates along a body axis, but that it restricts and orients an active axis-forming process which occurs later in the follicular epithelium or in the early embryo.

**Key words:** *Drosophila* pattern formation, oogenesis, pattern duplication, self-regulation, orthogonal axis orientation

**INTRODUCTION**

The analysis of maternally acting genes in *Drosophila* has revealed that a bidirectional interaction between soma and germ line precedes the establishment of embryonic dorsoventral polarity. The two steps in this interaction can be described as inductive processes in which a uniformly distributed receptor is locally activated via a ligand produced in a spatially restricted manner (for review, see Schüpbach et al., 1991; Chasan and Anderson, 1993).

The first of these inductive processes occurs during oogenesis and involves a dorsalizing signal produced in the germ line by the oocyte and received in the soma by the follicular epithelium which surrounds the oocyte (Schüpbach, 1987). The dorsalizing germ-line signal is probably the *gurken* (grk) protein which is homologous to TGF-α, a secreted growth factor (Neuman-Silberberg and Schüpbach, 1993). During mid stages of oogenesis, the oocyte nucleus moves to the future dorsal side of the oocyte (Spradling, 1993). At the same time, grk RNA becomes localized close to the nucleus in the dorsal-anterior corner of the oocyte. Presumably, the production of grk protein causes the local activation of the *Drosophila* EGF receptor homolog (*torpedo/DER*) which is expressed in all cells of the follicular epithelium (Price et al., 1989; Schejter and Shilo, 1989; R. Schweitzer, N. Zak, and B.-Z. Shilo, personal communication). This first process of induction leads to the establishment of a dorsoventral polarity in the follicular epithelium with dorsal follicle cells arising from a region of high *torpedo/DER* (top/DER) activation.

The follicle cells produce the two egg coverings, the chorion and the vitelline membrane. Both exhibit a dorsoventral polarity. The chorion, for example, bears dorsal appendages in dorsolateral positions (Fig. 1A). In addition to morphologically visible differences, the egg shell must also harbour molecular asymmetries guiding the second inductive process which establishes embryonic dorsoventral polarity. This conclusion is drawn from the fact that embryonic axis formation depends on an extraembryonic signal emanating from ventral regions of the perivitelline space (Stein et al., 1991; Roth, 1993). The spatial cues for this signal are provided by dorsal group genes whose function is required in the somatic tissue (Stein et al., 1991; Schüpbach et al., 1991). They probably cause the local activation of a protease cascade (Stein and Nüsslein-Volhard, 1992; Smith and DeLotto, 1994) which leads to the processing of *spätzle* protein (Morisato and Anderson, 1994). Processed *spätzle* protein is probably the ligand for the *Toll* receptor (Morisato and Anderson, 1994; Schneider et al., 1994) which itself is uniformly distributed in the plasma membrane of early embryos (Hashimoto et al., 1991). Local *Toll* activation leads to the spatially regulated nuclear import of *dorsal* protein. Thus, a nuclear concentration gradient of *dorsal* protein is established. *Dorsal* protein acts as a morphogen, regulating gene expression along the embryonic dorsoventral axis in a concentration-dependent manner (Steward, 1989; Rushlow et
al., 1989; Roth et al., 1989). This last step completes the transmission of spatial information: first, from the germ line to the soma and then back from the somatically derived egg coverings to the germ-line-derived embryo.

So far, little is known about how the two described inductive processes are linked. How much spatial information for the embryonic dorsoventral pattern is already contained in the localized activation of the top/DER receptor? To address this problem, we have undertaken a study of the embryonic phenotypes caused by mutations that affect either the strength or the spatial restriction of the dorsalizing germ-line signal.

MATERIALS AND METHODS

Fly stocks

The mutations used in this study have the following origins: top, grk$^{tr}$, grk$^{KG}$ (Schüpbach, 1987); grk$^{w2}$, top$^{+20}$, top$^{C3}$ (Clifford and Schüpbach, 1989); fs(1)K10$^d$ (Wieschaus, 1979); fs(1)K10$^{H515}$ (T. S. unpublished data); sqd$^{tr2}$ (T. S. unpublished data, Kelley, 1993); Df(3R)rad (Lindley and Zimm, 1992); orb$^{tr}$ (Christerson and McKearin, 1994); orb$^{E22}$ (Wieschaus and Nüsslein-Volhard, 1987); cact$^{tr2}$ (Roth et al., 1991).

In situ hybridization and antibody staining

To deal with extremely low fertilization rates caused by mutations affecting the egg shape, staged egg collections were covered with paraffin oil and cellular blastoderm embryos were hand-selected for fixation.

Transcripts were detected by in situ hybridization with digoxigenin-labeled DNA (dpp, sim, Dl) or RNA (rho) homologous to cDNA fragments of the corresponding genes. The hybridization procedure was a modification of the protocol of Tautz and Pfeifle (1989), using 55°C as the hybridization temperature in the case of RNA probes. Antibody stainings were done as described in Roth et al. (1989). Double stainings with antibodies and in situ hybridization probes were done according to Manoukian and Krause (1992). For the sectioning of ovaries, the same procedure was used as for embryos (Roth et al., 1989).

Chorion and cuticle preparations

For microscopic inspection of chorions and embryonic cuticular phenotypes, eggs were processed as previously described (Wieschaus and Nüsslein-Volhard, 1986).

RESULTS

To study how changes in the dorsalizing germ-line signal affect the embryonic dorsoventral pattern, we used mutations that either reduce signalling strength or alter the distribution of the signal. gurken (grk) and torpedo (top) mutations reduce the strength of the signal (Schüpbach, 1987). They lead to a ventralization of the egg shells. With increasing allelic strength, ventral regions of the egg shell expand at the expense of dorsal and lateral regions. This causes a fusion, reduction and finally deletion of the dorsal appendages (Fig. 1A,B,L,K). Mutations in fs(1)K10 (K10) (Wieschaus, 1979; Prost et al., 1988; Cheung et al., 1992) and squid (sqd) (Kelley, 1993) prevent the localization of grk RNA to the dorsal-anterior corner of the oocyte (Neuman-Silberberg and Schüpbach, 1993). Hence, the dorsalizing signal is not restricted. Weak phenotypes show both an enlargement and a shift of the dorsal appendages to the lateral side of the egg (Fig. 1C). In stronger phenotypes, the further enlargement of the dorsal appendages results in their fusion at the ventral side (Fig. 1D). Mutations in orb cause both a mislocalization and a reduction of the dorsalizing signal (Lantz et al., 1992; Christerson and McKearin, 1994; see below). Thus, the eggs have features of both dorsalization and ventralization and might result from an expansion of lateral egg shell regions (Fig. 1L,K, see Figure legend for explanation).

Changes in the dorsalizing germ-line signal do not significantly affect the slope of the dorsal morphogen gradient

The nuclear dorsal protein distribution along the embryonic circumference can be subdivided into three regions. A ventral plateau region with highest nuclear protein concentrations and a dorsal region without detectable nuclear protein are connected by a lateral region with decreasing concentrations. The size of the latter defines the slope of the gradient. The three regions correspond approximately to distinct expression domains of zygotic genes. twist (twi), a marker for the mesoderm, is expressed in a ventral region (constituting 25% of the embryonic circumference; Thissle et al., 1988; Fig. 2A). zerknäult (zen) and decapentaplegic (dpp) are expressed dorsally (40% of the embryonic circumference; Rushlow et al., 1987; St. Johnston and Gelbart, 1987; Fig. 2A). The region with intermediate nuclear dorsal protein concentrations shows neither zen (dpp) nor twi expression and its ventral half harbours the 8-cell-wide ventrolateral expression domain of rhomboid (rho, Bier et al., 1990; Ip et al., 1992; Figs 3A, 4B).

Embryos developing inside weakly ventralized egg shells show a strong expansion of the mesoderm. Weak top and grk mutations cause almost a twofold increase in the width of the twi domain (Table 1; Fig. 2A,B). The enlarged twi domain gives rise to a huge mass of mesodermal cells which is not enveloped by the remaining epidermis. Consequently, the embryo remains ventrally open (Figs 1F, 2D).

Despite the strong expansion of mesodermal anlagen, molecular markers for dorsal regions are still expressed. Both zen and dpp can be detected, but their expression domains have approximately half the width of the corresponding wild-type domains (Table 1; Fig. 2A,B,E). Since the reduction in size of the zen (dpp) domain corresponds to the increase in size of the twist domain, the region lacking zen and twist expression has the same width as in wild type, but is shifted to more dorsal positions of the embryonic circumference (Table 1; Fig. 2B). A preservation of the size of lateral anlagen can also be seen using rho as a marker. top and grk mutations do not change the width of the ventrolateral rho domain, but they shift the domain to more dorsal positions (Fig. 3). These data suggest that top and grk mutations lead to a nuclear dorsal protein distribution which has an increased ventral plateau region, but retains a normal slope.

We have demonstrated this directly for strong grk mutations, which cause a complete deletion of zen and dpp expression dorsally, but have rho domains of normal width strongly shifted towards the dorsal side (Fig. 4D). Such mutations lead to high nuclear dorsal protein concentrations in more than 60% of the ventral egg circumference (30% in wild type; Fig. 4A,B). However, the nuclear dorsal protein concentrations decrease rapidly towards the dorsal side. In wild-type embryos stained and sectioned together with grk embryos, we counted 14 nuclei
Fig. 1. Dark-field photographs of egg shells and cuticles. (A-D, I-M) Egg shells; (E-H) cuticles. Anterior is at left. (A,E) Wild type. (B,F) Weak ventralization caused by grk\textsuperscript{ED}/grk\textsuperscript{ED}, 98% of the eggs have one dorsal appendage (da) at the dorsal midline, 51% contain embryos. The normal appearance of the filzkörper (fk) indicates that the dorsolateral anlagen are not affected. There is a gap in the ventral epidermis. top\textsuperscript{top1}, top\textsuperscript{top1P2} and top\textsuperscript{top1}/top\textsuperscript{P2} cause similar phenotypes. (C,G) Weak dorsalization caused by sqd\textsuperscript{K12}/Df(3R)urd. 46% of the eggs have two enlarged da at lateral positions; 54% have fused da ventrally; 9% contain embryos. The embryos are dorsalized anteriorly. Half of them have reduced fk indicating a weak ventralization posteriorly. (D,H) Strong dorsalization caused by fs(1)K10\textsuperscript{K10}/fs(1)K10\textsuperscript{K10}. 84% of the eggs have fused da ventrally; <1% contain embryos. The embryos have either reduced or no fk and show an expansion of ventral epidermis (ve) posteriorly indicating a ventralization posteriorly. (L,M) Lateralization caused by orb\textsuperscript{mel}/orb\textsuperscript{F343}. The eggs are short and rounded posteriorly like dorsalized eggs. However, like ventralized eggs their dorsal appendages are fused dorsally (49% of the eggs) or they completely lack dorsal appendage material (46% of the eggs). This phenotype might result from an expansion of lateral regions of the follicular epithelium at the expense of dorsal regions. 37% of the eggs contain embryos. da, dorsal appendage; fk, filzkörper; de, dorsal epidermis; ve, ventral epidermis.
between highest and non-detectable nuclear concentrations. In grk embryos, an average of 13 nuclei was found indicating that the slope of the gradient is not significantly different from that of wild-type embryos.

Table 1. twist and zen expression in embryos mutant for torpedo or cactus

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Embryos simultaneously stained with zen and twi antibodies were sectioned. The total number of cells around the embryonic circumference (EC) and the number of twi- and zen-expressing cells were counted at selected positions along the anterior-posterior axis (%EL: % egg length, anterior tip = 100% EL). The maternal genotypes are: WT, Oregon R; cact, cact^A2/cact^A2; top, top^1/top^1.

Fig. 2. Ventral and dorsal anlagen of embryos derived from weakly ventralized eggs. (A-D) Transverse sections of embryos at the blastoderm stage (A,B), at gastrulation (C) or germ band extension (D). The maternal genotypes and molecular markers are: (A) wild type simultaneously stained with zen and twi antibodies; (B) top^1/top^1 simultaneously stained with zen and twi antibodies; (C) top^1/top^1F26 stained with twi antibodies; (D) top^1/top^1 stained with twi antibodies. (E) Dorsal surface view of a grk^ED22/grk^ED22 embryo at cellular blastoderm stage showing a stripe of dpp expression which has approximately half the width of the wild-type expression domain.

Fig. 3. rho expression in embryos derived from weakly ventralized eggs. Whole-mount preparations of embryos at cellular blastoderm stage showing the distribution of rho RNA. (A,B,C,E) Optical midsections. (D,F) Ventral surface views of the embryos shown in C and E. The maternal genotypes are: (A) wild type; (B-F) top^C1/top^C1. In wild type a ventrolateral and dorsal rho domain are visible. In mutant embryos, the dorsal rho domain is reduced or absent and the ventrolateral domain is shifted to more dorsal positions. The arrowheads demarcate the width of the ventrolateral rho domain. The embryos shown in C,D and E,F have a new rho domain ventrally. They have entirely lost the dorsal rho expression indicating that they are more severely ventralized than the embryo shown in B.
In summary, our data on weak and strong grk and top mutations reveal that they affect the dorsoventral morphogen gradient in a way drastically different from that of dorsal group and cactus mutations. The latter always change the slope of the gradient (for cactus see Table 1; Fig. 4E,F; Roth et al., 1989) while in grk and top a gradient with normal slope is shifted towards the dorsal side. A reduction in strength of the dorsalizing germ-line signal leads to pattern duplications in embryos resulting from weak grk and top mutations the increased mesodermal region invaginates forming two ventral furrows instead of one ventral furrow in wild type (Schüpbach, 1987; Fig. 2C). Costa et al. (1994) have shown that folded gastrulation, a gene that coordinates cell shape changes, shows highest levels of expression in distinct lateral regions in top embryos. This argues

A reduction in strength of the dorsalizing germ-line signal leads to pattern duplications

In embryos resulting from weak grk and top mutations the increased mesodermal region invaginates forming two ventral furrows instead of one ventral furrow in wild type (Schüpbach, 1987; Fig. 2C). Costa et al. (1994) have shown that folded gastrulation, a gene that coordinates cell shape changes, shows highest levels of expression in distinct lateral regions in top embryos. This argues

Fig. 4. dorsal protein distribution and rho expression in embryos derived from grk and cactus mutant females. Transverse section of embryos at blastoderm stage. (A,C,E) dorsal protein distribution. The arrows indicate regions with peak levels of the dorsal gradient. (B,D,F) rho RNA distribution. The maternal genotypes are: (A,B) Wild type. (C,D) grkHKGK /grkWG. (E,F)cactA /cactA. The grk embryo shows a deletion of the dorsal rho domain and a shift of the ventrolateral rho domains to the dorsal side. The shifted ventrolateral rho domain has a normal width. A new rho domain emerges ventrally (asterisk). In cactus embryos, in contrast, the ventrolateral rho domain is expanded to cover the entire embryonic circumference except the mesodermal region.

Fig. 5. Pattern duplication in embryos derived from strongly ventralized eggs. The maternal genotype is grkHKGK /grkWG. (A) Ventral surface view of embryo at early gastrulation stained with twi antibody. Anterior is left. (B) The transverse section through the embryo shown in A at 60% egg length reveals two distinct regions of twi expression. (C) Transverse section of embryo at germ band extension stage showing Dl RNA distribution. In wild type, Dl expression forms a ventral-to-dorsal gradient and is absent in the mesoderm. In grk embryos, Dl has a dorsal and a ventral domain of expression. (D) Transverse section of embryo at early gastrulation showing four regions of sim expression. They correspond to the borders of the two mesodermal regions.
Fig. 6. The anterior-posterior pattern of grk and K10 embryos. The embryos show both ftz protein (brown) and sim RNA (blue) distributions. (A) Optical midsection; (C,E) lateral surface view; (B,D,F) ventral surface view. (A,B) Wild-type embryo at gastrulation. (C,D) grkgrkgrk at cellular blastoderm. The embryo possesses four longitudinal stripes of sim expression. (E,F) fs(1)K10fs(1)K10 at cellular blastoderm. sim is expressed in a ring with an oblique angle relative to the ftz stripes.
for a pattern duplication within the enlarged mesodermal region which precedes the formation of the two ventral furrows.

Interestingly, a small number of *top* embryos (<1%) has a new zone of *rho* expression along the ventral midline. In some embryos, this zone is found as a small spot at around 70% egg length (Fig. 3C,D), but it can also appear as a more extended ventral stripe (Fig. 3E,F). The new ventral *rho* domain appears to reflect the formation of a new ventrolateral region along the ventral midline, which lies between the two ventral furrows. This interpretation is supported by the phenotypes caused by strong *grk* mutations.

In embryos derived from *grk* *HK*/*grk* *WG* females, the *twi* domain splits into two stripes (Fig. 5A,B). The zone separating the *twi* expression domains ventrally exhibits features of lateral anlagen. It expresses *rho* (Fig. 4D) and the neurogenic gene *Delta* (Fig. 5C; Vässin et al., 1987) indicating a juxtaposition of mesodermal and ventral ectodermal regions (Figs 5D, 6C,D). These data demonstrate a partial duplication of the dorsoventral pattern; two mesodermal regions in lateral positions are flanked dorsally and ventrally by neuroectoderm.

The pattern duplications result from a shape change of the nuclear *dorsal* protein gradient. As mentioned above, strong *grk* mutations lead to high nuclear *dorsal* protein concentrations in the ventral half of the embryo. Interestingly, within this ventral region, the *dorsal* protein concentrations are not even. They exhibit a decrease around the ventral midline and attain peak levels laterally (Fig. 4C). Thus, in contrast to wild type (Fig. 4A), the gradient has two maxima separated by a shallow ventral minimum. The decrease of nuclear *dorsal* protein concentrations ventrally corresponds to the formation of the new neuroectodermal region around the ventral midline.

In summary, a reduction in the strength of the dorsalizing germ-line signal causes a transition from a bell-shaped nuclear *dorsal* protein distribution (wild type) to a distribution with two maxima. The resulting pattern duplications along the dorsoventral axis, leave the anterior-posterior axis largely unaffected as can be seen from the expression pattern of the pair-rule gene *ftz* (Carroll and Scott, 1985) in strongly mutant *grk* embryos (Fig. 6C,D).

**A mislocalization of gurken RNA changes the orientation of the embryonic dorsoventral axis**

Cross sections through wild-type ovaries (stage 9 and 10) show *grk* RNA concentrated between the dorsally localized oocyte nucleus and the plasma membrane of the oocyte in a sector of the oocyte which abuts about 15% of the circumference of the follicular epithelium (Fig. 7B). In *K10* (Fig. 7C,D) and *sqd* (data not shown) egg chambers, the oocyte nucleus has a dorsal-anterior location, as in wild type, but *grk* RNA is visible at all positions around the oocyte circumference. The mislocalized RNA is confined tightly to the periphery of the oocyte and more RNA is present close to the oocyte nucleus. In *orb* mutant egg chambers *grk* RNA spreads to the ventral side of the oocyte (Christerson and McKearin, 1994). However, in contrast to *K10* and *sqd*, the RNA is no longer confined to the cell periphery, but is present in more central regions of the ooplasm (Fig. 7E,F). This distribution results in very low RNA concentrations at the cell periphery. Thus, we expect that *orb* leads to both a more uniform distribution of the dorsalizing signal and a reduction in its strength. This might explain why

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**Fig. 7. grk RNA distribution in wild-type and mutant ovaries.** (A,C,E) Optical midsections of stage 9 (A,C) or stage 10 (E) egg chambers. (B,D,F) Transverse sections through egg chambers at the position of the oocyte nucleus. (A,B) Wild type; (C,D) *fs(1)K10*/*fs(1)K10*; (E,F) *orb* mel/*orb* F343.
orb egg shells exhibit features of both ventralization and dor salization (Fig. 1L,M).

Embryos developing in dorsalized egg shells secrete no ventral cuticular structures at the anterior end indicating that they are dorsalized in this region. However, ventral denticles are present posteriorly (Wieschaus, 1979, Fig. 1G,H) and many embryos show signs of a ventralization at the posterior end. They exhibit a fusion or deletion of the dorsolaterally derived filzkörper and possess enlarged bands of ventral denticles (Fig. 1G,H). Thus, it seems that K10 and sqd embryos are dorsalized anteriorly and ventralized posteriorly.

This hypothesis is supported using molecular markers. In embryos from dorsalized egg shells, twi expression is absent along the anterior-posterior axis with the exception of a small patch anteriorly and a broad domain posteriorly (Fig. 8A). The posterior domain extends towards the dorsal side so that these embryos have more twi-expressing cells posteriorly than wild-type embryos. The ventrolateral rho expression domain forms an incomplete ring posteriorly instead of a longitudinal stripe in wild type. A broad rho expression domain encircling the entire embryo is found anteriorly which corresponds to the dorsal rho domain in wild type (Fig. 8B). dpp expression reveals the same fate map alteration (Fig. 8C, see also Figure legend).

Like the cuticle pattern, these patterns of dorsoventral gene expression reveal a coupling of anterior dorsalization and posterior ventralization. Thus, K10 and sqd embryos have a strong dorsoventral asymmetry along the anterior-posterior axis indicating that their dorsoventral axis is shifted so that it has a less than 90° angle to the anterior-posterior axis. The anterior-posterior axis of K10 and sqd embryos has a normal orientation as can be seen by the normal appearance of ftz expression stripes (Fig. 6E,F and data not shown). In the embryos stained for ftz protein, sim transcripts were visualized. The two parallel lines of sim expression in wild type, which are orthogonal to the ftz stripes (Fig. 6A,B), form a posterior circle in K10 (Fig. 6E,F). The plane of this circle has an oblique angle to the ftz stripes, directly demonstrating the altered orientation between both body axes.

A shift of the dorsoventral axis was also observed in orb embryos. orb embryos have extended twi domains posteriorly while twi expression is reduced or absent anteriorly (Fig. 8D). The ventral rho expression domain is not parallel, but obliquely oriented to the anterior-posterior axis (Fig. 8E). Thus, it forms an oblique stripe encircling the entire embryonic circumference. The plane in which the stripe resides intersects the anterior-posterior axis with varying angles.

orb embryos show not only a reduction of ventral, but also

Fig. 8. Dorsoventral axes with shifted orientation. Optical midsections of embryos at cellular blastoderm stage. (A) fs(1)K10¹/fs(1)K10¹; (B) fs(1)K10¹/fs(1)K10¹H35; (C) sqd¹²/Df(3R)urd. fs(1)K10¹/fs(1)K10¹H35 and sqd¹²/Df(3R)urd cause a similar phenotypic spectrum as fs(1)K10¹/fs(1)K10¹ with the exception that they lead to higher numbers of weak phenotypes (see also legend to Fig. 1). (D-F) orb¹⁰⁰| orb²₃⁴. (A,D) twi protein distribution; (B,E) rho RNA distribution; (C,F) dpp RNA distribution. For wild-type staining patterns see Figs 2 and 3. The expression domains of twi, rho and dpp are rotated with respect to the anterior-posterior axis. E.g. dpp in sqd embryos has a uniform expression along most of the embryonic axis indicating the presence of dorsal and dorsolateral fates in these regions. Posteriorly, a zone of dpp repression indicates the presence of ventral fates. The posterior-most expression of dpp is not under the control of the dorsoventral, but the terminal system, Ray et al. (1991).
of dorsal, anlagen anteriorly as visible from the small domain of dpp expression at the anterior tip (Fig. 8F). Therefore, they appear to have expanded dorsolateral anlagen at the anterior end. This is also obvious from the cuticle pattern of orb embryos (data not shown).

The transition from normal to shifted dorsoventral axes does not occur via a continuous change in the angle between the two body axes. Embryos from weakly dorsalized egg shells preserve normal dorsoventral anlagen anteriorly and posteriorly, but they exhibit a dorsralization in middle regions of the anterior-posterior axis (between 40% and 70% egg length; Fig. 9A-D). The more severe phenotypes show an extension of the dorsralized region towards anterior (Fig. 9E-H) until only a small patch of ventral pattern elements remains at the anterior tip. (Fig. 8A). Parallel to these changes, the dorsoventral pattern which is present posteriorly undergoes a shift in orientation. rho expression consists first of two branches which are only slightly tilted towards the anterior-posterior axis (Fig. 9B). This pattern gradually evolves into a straight line of expression which is orthogonal to the anterior-posterior axis and, thus, reflects an axis rotation by 90° as compared to wild type (Fig. 9G,H).

The fate map alterations of $K10$, sqd (data not shown) and orb embryos can be traced back to corresponding shape changes of the nuclear dorsal protein gradient. $K10$ embryos have detectable concentrations of nuclear dorsal protein in small anterior (data not shown) and a posterior region. While weaker phenotypes still show an asymmetric distribution with respect to the dorsoventral axis (Fig. 10A), in stronger phenotypes nuclear dorsal protein becomes symmetrically distributed (Fig. 10B). Thus, nuclear dorsal protein shows a posterior-to-anterior instead of a ventral-to-dorsal concentration gradient consistent with the dorsoventral axis being almost parallel to the anterior-posterior axis. In contrast to $K10$ and sqd, orb embryos possess a larger region with high nuclear dorsal concentrations posteriorly as expected from the pattern of zygotic gene expression (Fig. 10C; see also Christerson and McKearin, 1994).

It is noteworthy that, despite the shift in orientation, the dorsal protein gradients in $K10$, sqd and orb embryos have slopes similar to those of wild-type embryos. This can be seen directly in dorsal protein stainings (Fig. 10) or it can be inferred from the width of the ventrolateral rho domains (Fig. 8B,E). Thus, the preservation of the slope of the dorsal protein gradient appears to be a general feature of dorsoventral pattern defects caused by changes of the dorsalizing germ-line signal.

In summary, mislocalizations of grk RNA to the ventral side of the oocyte lead to embryonic dorsoventral axes which have a less than 90° angle with respect to the anterior-posterior axis. This suggests that the orthogonal orientation of the body axes depends on the proper anterior-dorsal localization of grk transcripts.

**DISCUSSION**

The analysis of embryonic dorsoventral pattern defects caused by alterations of the dorsalizing germ-line signal has yielded three main observations. (1) Neither a reduction nor a mislocalization of the signal leads to a significant change in the slope of dorsal protein gradients in $K10$, sqd, and orb embryos. (B.D.F.H) Ventral surface views of the embryos shown in A.C.E.G. All embryos show the distribution of rho RNA. The maternal genotypes are: (A,B) $K10^{th35}/K10^{th35}$; (C-H) sqd$^{D12}$/Df(3R)urd.
Our results have shown that one aspect of the embryonic dorsoventral morphogen gradient, namely its slope, is not significantly affected by changes in grk and top activities. Although it is not known how a complete lack of top/DER activity would affect the embryonic pattern, these data suggest that gradient formation occurs largely independent from the primary top/DER activation pattern. top/DER activation may only initiate the confinement of the region where the spätzle protein is processed. This region would then act as the source for a diffusion gradient of activated spätzle protein. Such a diffusion gradient would always have the same slope wherever the source region is established. For the purpose of our further discussion, we refer to this source region as the ventral prepattern.

To yield gradients of defined slope, the ventral prepattern must have a sharp border and the formation of this border must be possible even if top/DER activation is reduced (grk and top mutations) or spatially misplaced (K10, sqd and orb mutations). Thus, we expect that the primary pattern of top activation leads to a process of pattern refinement. This could occur by an enhancement of top/DER activation or its downstream processes at the dorsal side of the egg chamber. rho protein which is expressed in dorsal follicle cells was implicated in a positive feedback loop which regulates the spatial activation of top (Ruohola-Baker et al., 1993).

Further refinement processes could also take place on the ventral side. top/DER activation would repress a pattern formation process dorsally thereby confining its action to the ventral side (Fig. 11). The described ventral pattern duplications strongly argue for the existence of a ventrally located pattern formation system. This system could either act in the ventral follicular epithelium as proposed in Fig. 11 or it could result from positive feedback mechanisms occurring at the level of spätzle processing (e.g. interaction of serine proteases).

The establishment of the source for Toll ligand production employs lateral inhibition

If the embryonic dorsoventral morphogen gradient were simply complementary to the top/DER activation pattern in the follicular epithelium, then the reduction of top/DER activation should yield a uniform expansion of the mesoderm. Instead, we see a striking internal pattern duplication in the mesodermal region resulting in the formation of two ventral furrows and, in stronger phenotypes, in a splitting of the mesoderm accompanied by the formation of a new neurogenic region along the ventral midline. These pattern duplications must reflect corresponding changes of the Toll activation pattern, since they can be traced back to a change of the dorsal protein gradient. Thus, the loss of spatial information in the dorsal follicular epithelium does not simply result in a loss of spatial information for the embryonic axis, but causes in fact a de novo generation of pattern in the expanded ventral domain.

This phenomenon is similar to the formation of spacing patterns by lateral inhibition. For example, if during growth in the blue-green alga Anabaena two H cells have exceeded a specific distance, a new H cell differentiates at the location of largest distance from the preexisting cells. It is assumed that the H cells produce a diffusible inhibitor that suppresses H cell development in neighbouring cells (Wilcox et al., 1973). We propose that the establishment of the ventral prepattern is accompanied by a lateral inhibition process which limits its size. The inhibition suppresses the formation of more ventral
Localization and embryonic pattern

If the region where ventral prepattern formation can take place is enlarged, the lateral inhibition causes a splitting of the pattern. This mechanism could act either in the follicular epithelium (Fig. 11) or at the level of the serine protease cascade in the perivitelline space. It is however likely that no lateral inhibition occurs at the level of Toll activation or downstream of Toll (Roth, 1993).

Lateral inhibition mechanisms have so far mainly been used to describe the formation of point-like patterns which, in the best understood cases, can be traced back to the interaction of one cell with its nearest neighbours (for review see Greenwald and Rubin, 1992). However, theoretical studies have shown that under certain conditions, lateral inhibition mechanisms are compatible with the formation of spatially extended stripe-like patterns (Meinhardt, 1989).

**grk localization establishes the orthogonal orientation of the body axis**

*grk* RNA is concentrated in a small patch in the anterior–dorsal corner of the oocyte and, thus, exhibits not only a dorsoventral, but also an anterior–posterior asymmetry. Therefore, the geometry of *grk* RNA distribution appears to be different from that of the embryonic dorsoventral pattern: it is point-like as opposed to the stripe-like geometry underlying the embryonic pattern.

Currently, we do not know when the patterning processes occur in the follicle cell which orient the embryonic axis. Given the more spherical geometry of the early egg chamber, the anterior–dorsal secretion of *grk* protein might cause a radial pattern of top/DER activation in the follicular epithelium, which in turn would confine the ventral prepattern within the half-circular region with the largest distance from the center of top/DER activation. In this context, it is interesting to note that the embryonic dorsoventral pattern itself has an almost half-circular shape which becomes apparent if the terminal pattern formation system is removed (Fig. 12; Ray et al., 1991). Thus, this pattern could at least partially be derived from a half-circular prepattern in the follicular epithelium (Fig. 12).

However, other mechanisms may contribute to an elongation of the ventral prepattern. First, active mechanisms could operate in the cytoplasm or the extracellular space between oocyte and follicular epithelium leading to a more stripe-like distribution of *grk* protein. Second, a stripe-like activation of top/DER could result from the anterior-to-posterior movement of the follicle cells during stage 8 and 9 of ovarian development (Spradling, 1993).

If the point-like source for *grk* protein leads to a half-circular ventral prepattern, the axis shifts caused by *K10*, *sqd* and *orb* mutations can be partially explained. In *K10* and *sqd*, the spreading of *grk* RNA towards the ventral side might be accompanied by reduced RNA amounts dorsally. **top**/**DER**
activation would then increase ventrally and decrease dorsally. Consequently, the ventral prepattern forming system would be repressed ventrally and (slightly) derepressed dorsally. Thus, its orientation would shift from a ventral half-circular position in wild type to a posterior zone in K10 and sqd ovaries resulting in a reorientation of the embryonic dorsoventral axis (Fig. 12B).

Shifts of the embryonic dorsoventral axis are also generated by mislocalized grk RNA if the strength of top/DER signalling is reduced. This is seen in the phenotype of orb mutations, which show a loss of dorsal follicle cell fates (reduced top/DER activation) and a rotation of the embryonic dorsoventral axis. Apparently, the orientation of the embryonic dorsoventral axis depends not on the precise amount, but on the degree, of dorsoventral asymmetry exhibited by a given grk RNA distribution. This might also explain why even strong grk mutations do not affect the proper orientation of the embryonic dorsoventral axis. The system that establishes the ventral prepattern apparently enhances weak spatial asymmetries.

The model presented for the axis shift does not yet explain why strong K10 embryos have a small patch of twi expression anteriorly and it can also not account for the weak phenotypes. However, an explanation for these phenomena can be given invoking the local activation/lateral inhibition properties that we have used above to describe the mesoderm splitting (Roth et al., unpublished data).

Conclusions
The relationship between localized grk RNA and embryonic pattern is strikingly different from the way that the other localized RNAs determine parts of the body plan. Like grk RNA, bcd and nanos RNAs are localized in the oocyte. However, they act directly in the embryo (St. Johnston and Nüsslein-Volhard, 1992) while grk acts through the follicular epithelium. bcd and nanos RNAs are required for the differentiation of a specific part of the anterior-posterior axis. In contrast, grk is not responsible for the differentiation of any specific part of the embryonic dorsoventral axis. Complete dorsoventral gradients form if grk-dependent signalling is reduced or mislocalized. Instead of specific dorsoventral fates, grk defines the size and orientation of the domain in which axis formation can occur.

The process of axis formation employs self-enhancement and lateral inhibition. Thus, the spatial information of the embryonic dorsoventral morphogen gradient is not present in the grk protein distribution or the primary pattern of top/DER activation, but rather is generated de novo by a self-patterning process, which takes place either in the follicular epithelium or in the perivitelline space.

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