

Polarization of both major body axes in *Drosophila* by *gurken-torpedo* signalling

Acalmo González-Reyes, Heather Elliott & Daniel St Johnston

The Wellcome/CRC Institute and Department of Genetics, University of Cambridge, Cambridge CB2 1QR, UK

Anterior-posterior polarity in *Drosophila* arises from the movement of the oocyte to the posterior of the egg chamber, and the subsequent acquisition of posterior fate by the adjacent somatic follicle cells. We demonstrate that *gurken* is necessary in the oocyte and *torpedo/DER* in the follicle cells for the induction of posterior fate. As the *gurken-torpedo/DER* pathway also establishes dorsoventral polarity later in oogenesis, *Drosophila* uses the same germline to soma signalling pathway to determine both embryonic axes.

THE polarities of both the anterior-posterior (AP) and dorsal-ventral (DV) axes of *Drosophila* are determined during oogenesis as a result of inductive interactions between the germline cells of the egg chamber, the oocyte and the nurse cells, and the surrounding layer of somatic follicle cells (for a review of oogenesis see ref. 1). The first visible sign of AP asymmetry is the movement of the oocyte to the posterior of the nurse cells. Once the oocyte has reached this position, it induces the adjacent polar follicle cells to adopt a posterior fate rather than the default anterior fate². Later in oogenesis, the posterior follicle cells signal back to the oocyte to polarize its microtubule cytoskeleton, so the 'minus' ends lie at the anterior pole and the 'plus' ends at the posterior, thereby directing the microtubule-dependent localization of *bicoid* (*bcd*) and *oskar* (*osk*) messenger RNAs to opposite poles of the oocyte^{2–10}. Because *bcd* mRNA encodes the anterior determinant, and the localization of *osk* mRNA defines the site of formation of pole plasm, which contains the posterior and germline determinants^{11,12}, the specification of the posterior follicle cells defines the AP polarity of the resulting embryo. Thus AP axis formation requires two sequential inductions, first from the germ line to the somatic follicle cells, and then from the follicle cells back to the oocyte. However, the signalling molecules responsible for these two inductions have not yet been identified.

The formation of the DV axis of the embryo involves a similar series of inductions between the germ line and the follicle cells. During stages 8 and 9 of oogenesis, the oocyte induces the follicle cells on one side of the egg chamber to adopt a dorsal fate¹³. This induction requires the activity of *gurken* (*grk*) in the germ line and *torpedo/DER* (*top/DER*) in the follicle cells¹³. As *grk* encodes a transforming growth factor (TGF)- α -like protein that contains an epidermal growth factor (EGF) repeat¹⁴, while Top/DER is the *Drosophila* homologue of the EGF receptor^{15,16}, it has been proposed that the Gurken protein binds to the Top/DER protein in the adjacent follicle cells to activate a receptor tyrosine kinase signal transduction pathway that results in the determination of dorsal fate^{14,17,18}. The resulting polarization of the follicle cell layer determines the embryonic DV axis, as the ventral follicle cells will subsequently signal back to the germ line to define the high point of the dorsal nuclear gradient on the ventral side of the embryo¹⁹.

Although embryos laid by *grk* mutant females have been reported not to have AP defects²⁰, the initial description of the mutant phenotype suggested that *grk* might also be involved in the generation of AP polarity in the follicle cell layer¹³. In wild-type egg chambers, the anterior follicle cells produce a distinct structure called the micropyle at the anterior end of the egg, and the posterior follicle cells secrete the aeropyle. However, the eggs laid by *grk* mutant mothers often possess a second micropyle at the posterior pole¹³. Here we show that this phenotype is a result

of a duplication of anterior follicle cells at the posterior pole of the egg chamber. Because the AP phenotype of *grk* mutants is dependent on the germ line, we propose that *grk* encodes the inductive signal produced by the oocyte to determine posterior follicle cell fate. As a consequence of the lack of posterior follicle cells, the oocyte microtubule cytoskeleton develops a symmetric organization that results in the localization of *bcd* mRNA to both poles of the oocyte, and of *osk* mRNA to the centre. Because reduction of *top/DER* activity in the follicle cells gives rise to the same phenotype, we conclude that Gurken signals to Top/DER to establish the polarity of the AP axis. The polarization of the oocyte cytoskeleton is required for the subsequent microtubule-dependent movement of the germinal vesicle to the dorsal anterior margin of the oocyte. As the position of the germinal vesicle determines the site of localization of *grk* mRNA, and hence where the dorsal follicle cells are induced, the formation of the DV axis depends on the earlier polarization of the AP axis.

AP polarity requires *grk*

Several subpopulations of follicle cells can be distinguished along the AP axis of wild-type egg chambers¹ (Fig. 1A–E). At stage 9 of oogenesis, a cluster of follicle cells at the anterior tip of the egg chamber, the border cells, start to express the *slow border cells* (*slbo*) gene and delaminate from the follicle cell layer to migrate between the nurse cells to the anterior margin of the oocyte²¹ (Fig. 1C). The adjacent anterior follicle cells become stretched to cover the nurse cells, as the rest of the follicle cells migrate posteriorly to form a columnar epithelium around the oocyte. Later, the anteriormost columnar follicle cells migrate centripetally between the nurse cells and the oocyte (Fig. 1A, B). These three anterior populations of follicle cells, the border cells, the stretched follicle cells and the centripetal cells, all express the L53b β -gal enhancer trap line²² (Fig. 1D). At stage 13 of oogenesis, the posterior follicle cells express the 12A3 β -gal fusion construct²³ (Fig. 1Ea, Ec).

In *grk* mutants, all three anterior follicle cell types are duplicated at the posterior end of the egg chamber. A small cluster of *slbo*-expressing cells form at the posterior pole, and a larger group of posterior cells activate the L53b enhancer trap (Fig. 1H, I). Furthermore, these 'posterior' cells undergo the same morphological movements as the anterior follicle cells: the *slbo*-expressing cells lose their epithelial organization to form a ball of cells, the adjacent cells become stretched, and the neighbouring columnar cells migrate centripetally, sometimes bisecting the oocyte (Fig. 1F, G). Thus the border cells, stretched follicle cells and the centripetal cells autonomously perform their normal developmental programmes when they are duplicated in reverse order at the posterior of *grk*[–] egg chambers, even though they are no longer in contact with the nurse cells. The duplication of

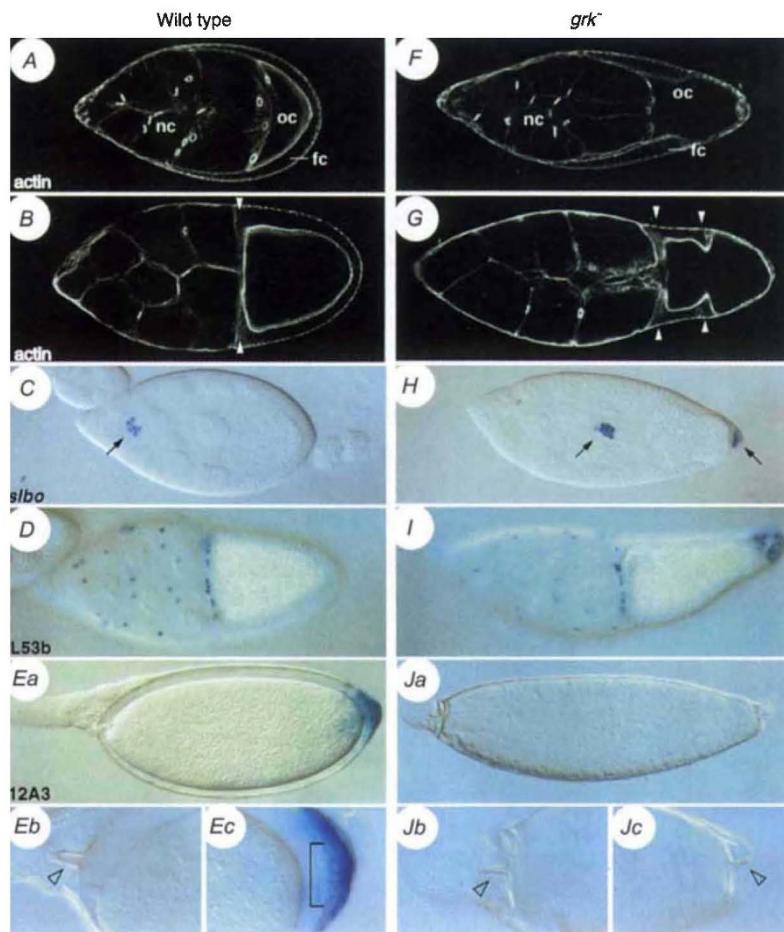


FIG. 1 The induction of posterior follicle cell fate requires *grk*. *A*, *B*, *F*, *G*, Rhodamine-labelled phalloidin staining of actin filaments. *C*–*E*, *H*–*J*, X-gal-stained egg chambers. An egg chamber consists of a layer of somatic follicle cells surrounding 15 nurse cells and a posterior oocyte which are interconnected by actin-rich ring canals¹. At stage 9, most of the follicle cells adopt a columnar shape and migrate posteriorly to envelop the oocyte (*A*). By stage 10, the anteriormost columnar follicle cells migrate centripetally between the nurse cells and the oocyte (*B*). Anterior to these lie the border cells, which express an enhancer trap in the *slbo* gene at stage 9 (*C*), and the adjacent stretched follicle cells. The enhancer trap line L53b expresses in the border cells, the stretched follicle cells and centripetal follicle cells at stage 10 (ref. 22) (*D*). At stage 14, the border cells and centripetal follicle cells make the micropyle (*Ea*; magnified in *Eb*), while the posterior follicle cells, which express the β -gal construct 12A3 (ref. 23) (*Ea*), secrete the aeropyle (*Ec*). *F*–*J*, All of the *grk*^{2E12}/*grk*^{2B6} egg chambers show a duplication of all three anterior follicle cell types at the posterior pole. The follicle cells at the posterior migrate anteriorly at stage 9 (*F*), some stretch over the oocyte and others migrate centripetally at stage 10 (*G*). There is expression of *slbo* at the posterior at stage 9 (*H*) and L53b at stage 10 (*I*), but 12A3 expression is absent (*J*). These ectopic anterior follicle cells secrete a second micropyle at the posterior pole of the egg, in place of the aeropyle (*Ja*, *b*, *c*). Abbreviations and symbols: fc, follicle cells; nc, nurse cells; oc, oocyte; arrowheads, centripetal follicle cells; arrows, border cells; open arrowheads, micropyle; bracket, aeropyle. In all figures, anterior is to the left and dorsal up.

anterior fates at the expense of posterior follicle cell fates is also apparent later in oogenesis. The follicle cells at the posterior pole do not express the posterior marker 12A3 and produce a second micropyle instead of the aeropyle (Fig. 1*J*). The changes in follicle cell fates in mutant egg chambers indicate that *grk* is required for the induction of posterior follicle cell fate by the oocyte.

To distinguish whether *grk* activity is required for the production of the inductive signal by the oocyte or for the reception of this signal in the somatic follicle cells, pole cells were transplanted to generate chimaeric egg chambers in which the oocyte and nurse cells are mutant for *grk*, but the follicle cells are *grk*⁺. Removal of *grk* in the germline cells induces ectopic expression of the anterior follicle cell marker L53b in the wild-type follicle cells at the posterior of the egg chamber (Fig. 2). Thus *grk* is required in the germ line for the induction of posterior follicle cell fate, strongly suggesting that it encodes the posterior inducer. Because *grk* mRNA is localized to the posterior margin of the oocyte during the early stages of oogenesis¹⁴, it is likely that the export of Gurken is polarized towards the responding polar follicle cells.

AP polarity in the oocyte

Later in oogenesis, the posterior follicle cells signal back to the germ line to determine AP polarity within the oocyte. If this signalling fails to occur, either as a result of loss of *Notch* or *Delta* activity in the follicle cell layer, or the absence of protein kinase A in the germ line, the oocyte develops a duplicated AP axis^{3,4}. The *grk*[−] egg chambers, which lack posterior follicle cells, also show a mirror-image duplication of the AP axis of the oocyte. Both *bcd* and *K10* mRNAs²⁴, which normally localize to just the anterior pole of the oocyte, become localized to both poles, and *osk* mRNA and Staufen protein²⁵ localize to the centre rather than the posterior (Fig. 3 and data not shown).

Because the localization of *bcd* and *osk* mRNAs is microtubule dependent^{6,8}, their altered distributions in *grk*[−] egg chambers are likely to reflect a change in the organization of the oocyte microtubule cytoskeleton. To test this possibility, we used a kinesin- β -galactosidase fusion protein as a marker of microtubule polarity²⁶. This chimaeric protein contains the motor domain of the plus-end-directed microtubule motor kinesin, and accumulates at the plus ends of the microtubules at the posterior of wild-type stage 9 oocytes⁶. In *grk*[−] egg chambers, the fusion protein localizes to the centre of the oocyte, in the same position as *osk* mRNA (Fig. 3). This indicates that the oocyte microtubule network has a symmetric organization, with the minus ends at both poles and the plus ends in the centre. Thus the induction of the posterior follicle cells by *grk* is required for the polarization of the oocyte cytoskeleton, and hence the generation of the AP axis of the embryo. Because *bcd* and *osk* mRNA localization begins at stage 7, Gurken must induce posterior fate before this stage.

AP polarity requires *top/DER*

During early oogenesis, *top/DER* mRNA is expressed throughout the follicle cell layer²⁷. We therefore investigated whether *top/DER* is required in these cells for the reception of the Gurken signal, as it is in the dorsal follicle cells later in oogenesis. Because *top/DER* is required at several stages of development²⁸, it is only possible to examine the maternal phenotype of hypomorphic mutant combinations that survive to adulthood. Nevertheless, a large proportion of such mutant egg chambers develop an AP phenotype very similar to that produced by *grk* mutations. The follicle cells at the posterior of the egg chamber behave abnormally and express anterior markers such as L53b, and the polarity of the oocyte itself is also affected: *bcd* mRNA localizes to both poles, and *osk* mRNA localizes to the centre (Fig. 4*A*). Because *top/DER* is not required in the

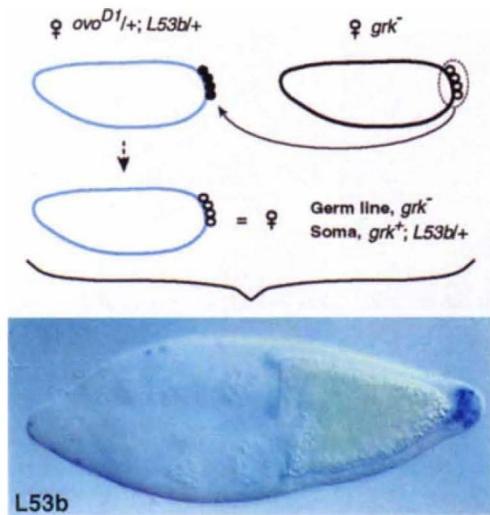
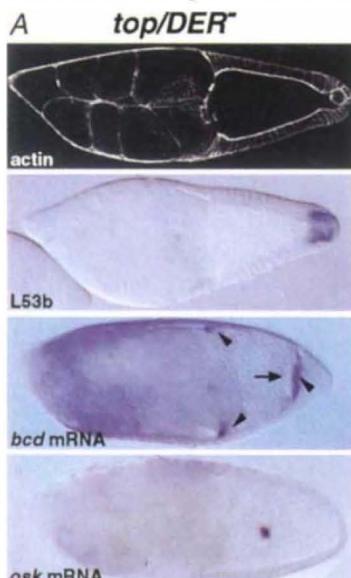


FIG. 2 The determination of posterior follicle cell fate requires *grk* in the germ line. In chimaeric egg chambers in which the germ line is mutant for *grk* and the follicle cells are wild type, the anterior marker L53b is expressed at the posterior of the follicle cell layer. These follicle cells also undergo the same morphological changes as the anterior follicle cells and produce a second micropyle.

METHODS. Males of the genotype *ovo*^{D1}/Y were crossed to females carrying the L53b enhancer trap line to generate host embryos of the genotype *ovo*^{D1}/+; L53b/+⁺. Eggs laid by *grk*^{2B6}/CyO flies (a putative null allele of *grk* (ref. 14)) were used as donor embryos. When female pole cells are transplanted into female embryos, one quarter of the chimaeric egg chambers have a germ line which is mutant for *grk*, surrounded by wild-type follicle cells which carry the L53b enhancer trap line.

germ line^{13,29}, this phenotype is presumably a consequence of the lack of gene activity in the follicle cell layer, strongly suggesting that Top/DER is the receptor for Gurken in the posterior follicle cells.

When Top/DER protein is activated at other stages of *Drosophila* development, it initiates a typical receptor tyrosine kinase signal transduction pathway that leads to the activation of the mitogen-activated protein kinase (MAPK), encoded by *rolled* (*rl*)^{30,31}. We therefore examined whether the removal of one copy of *rl* would enhance the phenotype of a weak *top/DER* mutant combination, *top*^{CA}/*top*^{QY1}, in which *osk* mRNA always localizes to the posterior of the oocyte. In 100% of *rl*^{10A}



10 egg chambers with expression of L53b at the posterior (3.3 stained cells on average), and 74% of stage 9 egg chambers displaying a mutant *osk* mRNA localization (60% weak phenotype and 14% strong). *top*^{QY1}/*top*^{CO} stage 10 egg chambers gave 100% expression of L53b at the posterior (22.5 cells on average); 31% of stage 9 egg chambers showed a weak, and 36% a strong, *osk* mRNA mislocalization. *B*, Of *cni*^{AR55}/*cni*^{AA12} mutant egg chambers, 46.5% show L53b staining at the posterior (3.25 cells on average); in 32.8% of stage 9 oocytes, *osk* mRNA is mislocalized to the middle.

METHODS. *cni* mutant egg chambers were *cni*^{AR55}/*cni*^{AA12} (ref. 36); *orb* mutant egg chambers were *orb*^{mel}/*orb*^{F343} (refs 33, 34). Other strains used are: *capu*^{G7}, *K10*, *rl*^{10A}, *top*^{CA}, *top*^{CO}, *top*^{QY1}, *spir*^{RP} (refs 15, 30, 32, 35).

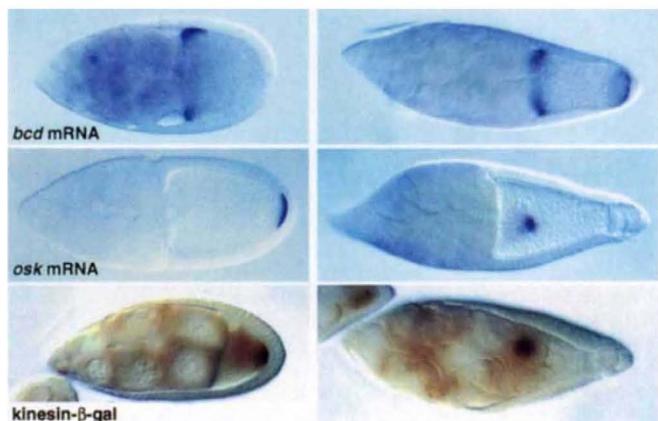


FIG. 3 The polarization of the AP axis of the oocyte requires *grk*. In wild-type egg chambers, *bcd* mRNA localizes to the anterior margin of the oocyte. In 67% of *grk*⁻ egg chambers, *bcd* mRNA localizes to both poles of the oocyte. In wild-type egg chambers, *osk* mRNA to the posterior pole. In 67% of *grk*⁻ egg chambers, *bcd* mRNA localizes to both poles of the oocyte, and *osk* mRNA localizes to the centre of the ooplasm in 87% of the mutant oocytes. *osk* mRNA normally accumulates at the posterior of the oocyte, and 87% of *grk*⁻ oocytes show *osk* mRNA localized to the centre of the ooplasm. The chimaeric protein kinesin- β -gal⁶ concentrates at the posterior of stage 9 wild-type oocytes. In *grk*⁻ oocytes, the kinesin- β -gal protein accumulates in the middle.

METHODS. *In situ* hybridizations and actin staining (confocal microscopy) was used to collect the images from different focal planes as described². Staining using β -galactosidase was as described in ref. 21, and immunohistochemistry in ref. 25. We used the transgenic line KZ503 (ref. 6) to express the kinesin- β -gal protein in the oocyte. Although all the allelic combinations tested gave the *grk*⁻ phenotype (*grk*^{WG}/*grk*^{WG}, *grk*^{HK}/*grk*^{HK}, *grk*^{HK}/*grk*^{WG}, *grk*^{2B6}/*grk*^{2B6}, *grk*^{2E12}/*grk*^{2B6}, *grk*^{2B6}/*grk*^{HK} and *grk*^{2E12}/*grk*^{HK} (refs 13, 14)), all the results shown correspond to *grk*^{2E12}/*grk*^{2B6} egg chambers.

top^{CA}/*+top*^{QY1} egg chambers, *osk* mRNA accumulates in the centre of the oocyte. This strong dominant enhancement of the *top/DER* phenotype by *rolled* suggests that the MAPK pathway is involved in the induction of posterior follicle cell fate.

AP polarity requires *cornichon*

The discovery that *grk* and *top/DER* are required for the induction of posterior follicle cell fate raised the possibility that other

FIG. 4 Role of *top/DER* and *cni* in the establishment of AP polarity. *A*, The phenotype of *top*^{QY1}/*top*^{CO} egg chambers resembles that of *grk*⁻: the posterior follicle cells show an abnormal shape and behaviour and express L53b; *bcd* mRNA accumulates at both poles of the oocyte (arrowheads) and *osk* mRNA localizes to the centre of the oocyte. The arrow points to the germinal vesicle at the posterior of the oocyte. In addition to the expression of the L53b β -gal line, we have also scored *osk* mRNA localization as a measure of the strength of the phenotype and established two categories: weak cases show an incomplete and diffuse posterior localization of *osk* mRNA; in strong cases *osk* mRNA accumulates in the centre of the oocyte. The weak combination *top*^{QY1}/*top*^{QY1} gave rise to 54% of stage

stage 9 egg chambers with expression of L53b at the posterior (3.3 stained cells on average), and 74% of stage 9 egg chambers displaying a mutant *osk* mRNA localization (60% weak phenotype and 14% strong). *top*^{QY1}/*top*^{CO} stage 10 egg chambers gave 100% expression of L53b at the posterior (22.5 cells on average); 31% of stage 9 egg chambers showed a weak, and 36% a strong, *osk* mRNA mislocalization. *B*, Of *cni*^{AR55}/*cni*^{AA12} mutant egg chambers, 46.5% show L53b staining at the posterior (3.25 cells on average); in 32.8% of stage 9 oocytes, *osk* mRNA is mislocalized to the middle.

genes involved in DV axis formation might also participate in this process. We therefore examined the expression of the anterior follicle cell markers, L53b or *slbo*, in: (1) *cappuccino*, *spire* and *orb* mutants which, like *grk*, affect the polarity of both axes^{32,34}; (2) *K10* mutants, which cause a dorsalized phenotype

as a result of the failure to localize *grk* mRNA to the dorsal side of the oocyte nucleus^{14,35}; and (3) the ventralizing mutant *cornichon* (*cni*)^{36,37}. Whereas mutations in *cappuccino*, *spire*, *orb* and *K10* do not affect posterior follicle cell determination, mutants in *cni* result in very similar AP defects to those seen in *grk*⁻

FIG. 5 Mislocalization of the germinal vesicle in *grk* and *top*/DER mutant egg chambers. A, B Hoechst staining to visualize DNA. C, D, *grk* mRNA localization. A, The germinal vesicle lies at the dorsal-anterior corner of wild-type stage 8 and older oocytes. B, In 31% of stage 8 or older *grk*⁻ oocytes, the germinal vesicle lies at the posterior. This phenotype is seen in 5–10% of *top*^{OY}/*top*^{CO} egg chambers. C, In wild-type stage 9 oocytes, *grk* mRNA localizes above the germinal vesicle in the dorsal-anterior corner. D, When the germinal vesicle fails to migrate, as in this *top*^{OY}/*top*^{CO} oocyte, *grk* mRNA remains localized to the posterior pole.

METHODS To visualize DNA, we used the DNA dye Hoechst as described².

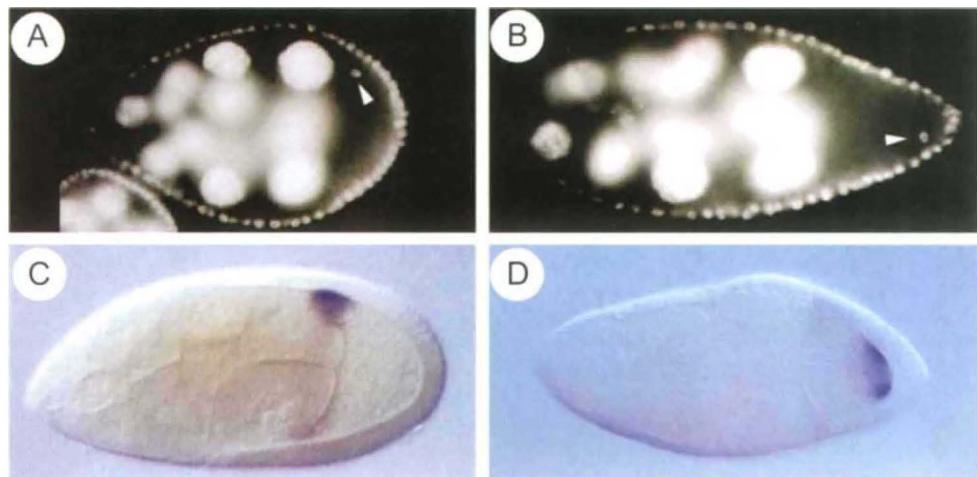
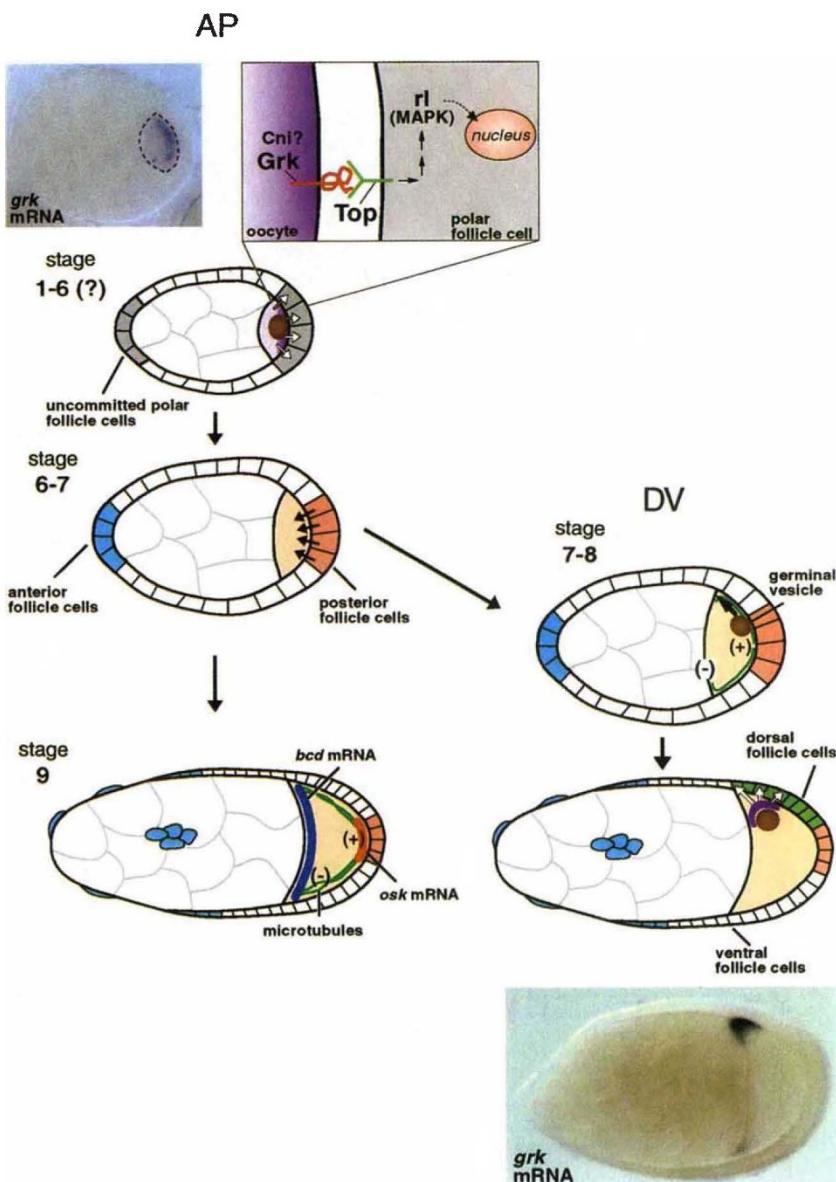


FIG. 6 A model for the generation of AP and DV polarity in *Drosophila*. AP polarity arises from the movement of the oocyte to the posterior of the nurse cells². The germinal vesicle and *grk* mRNA both become localized to the posterior of the oocyte, leading to the polarized production of extracellular Gurken. This then binds to Top/DER in the adjacent uncommitted polar follicle cells to activate a receptor tyrosine kinase signalling pathway to determine posterior follicle cell fate. The posterior follicle cells subsequently signal back to the oocyte to repolarize the oocyte microtubule cytoskeleton, probably by inducing the disassembly of the microtubule organizing centre at the posterior of stage 6 oocytes⁵. The generation of a new microtubule nucleating activity at the anterior margin of the oocyte thus creates a polarized microtubule network, which directs the localization of *bcd* and *osk* mRNAs to opposite poles of the oocyte, thereby defining the AP axis of the embryo. This repolarization of the oocyte cytoskeleton also determines the orientation of the DV axis, because it is required for the microtubule-dependent movement of the germinal vesicle to a site at the anterior margin of the oocyte^{5,38}. The position of the germinal vesicle directs the subsequent relocalization of *grk* mRNA to the dorsal anterior corner of the oocyte¹⁴. The resulting localized synthesis of Gurken induces the follicle cells on this side of the egg chamber to adopt a dorsal fate, as they migrate from the nurse cells to cover the oocyte. Thus the sequential induction of posterior and dorsal follicle cell fates by localized Gurken establishes first AP and then DV polarity.



egg chambers. In nearly half of *cni* mutant egg chambers, L53b is expressed in the posterior follicle cells and *osk* mRNA is mislocalized to the centre of the oocyte (Fig. 4B). Thus, like *grk* and *top/DER*, *cni* is required for the induction of both posterior and dorsal follicle cell fates. Because the ventralized phenotype of *cni* mutants is germline dependent³⁷, Cornichon is probably required in the oocyte for the production of the inducing signal. However, *cni* mutants do not affect the localization of *grk* mRNA to the posterior of the oocyte (data not shown), suggesting that it must act at a different step in this pathway.

AP is the primary axis

During stages 7 and 8 of oogenesis, the germinal vesicle migrates from the posterior of the oocyte to the anterior margin of the cell, in a process that requires microtubules^{5,38}. Because the absence of posterior follicle cells in *grk* and *top/DER* mutants prevents the polarization of the oocyte microtubule cytoskeleton, we examined whether these mutants also affect the positioning of the germinal vesicle. In about one-third of *grk* (or 5 to 10% of *top/DER*) mutant egg chambers, the germinal vesicle fails to migrate to the anterior margin (Fig. 5A, B). This result is most easily explained if the germinal vesicle moves towards the 'minus' ends of the microtubules, which lie at the anterior pole in wild-type oocytes. In the mutant egg chambers where the cytoskeleton develops a symmetric organization, with the 'minus' ends of the microtubules at both poles, the germinal vesicle can localize to either end of the oocyte.

In a wild-type egg chamber, *grk* mRNA accumulates above the germinal vesicle after it has migrated to the anterior of the oocyte¹⁴. In the *top/DER* mutant egg chambers in which the germinal vesicle has failed to migrate, *grk* mRNA also remains localized to the posterior pole of the oocyte (Fig. 5C, D). This suggests that the germinal vesicle directs the localization of the mRNA. As the localization of *grk* mRNA serves to restrict where Gurken signalling induces dorsal follicle cell fate, the orientation of the DV axis is determined by the position of the germinal vesicle, which in turn requires the polarization of the oocyte cytoskeleton that is induced by the posterior follicle cells. Thus the formation of the DV axis depends on the prior polarization of the AP axis.

Discussion

We have previously proposed that AP polarity in *Drosophila* arises when the oocyte moves to the posterior of the egg chamber and induces the adjacent follicle cells to adopt a posterior fate². The results presented here confirm this view, and lead us to propose the model for AP axis formation shown in Fig. 6. One surprising aspect of these results is the discovery that Gurken,

Top/DER and Cornichon are required for the induction of both the posterior and dorsal follicle cells, and hence for the polarization of both the AP and DV axes. This raises the question of how the same signalling pathway can induce two different follicle cell states. Several enhancer trap lines and antibodies label a subset of the follicle cells at each pole of the egg chamber from early oogenesis onwards, suggesting that the polar follicle cells have been specified as distinct from the rest of the follicle cell population before either induction takes place^{3,22,39,40}. It therefore seems likely that the two follicle cell populations differ in their competence to respond to the inductive signal: the fate of the polar follicle cells is restricted to a choice between anterior or posterior, and the rest of the follicle cells are committed to become dorsal or ventral. If this is correct, the orientation of the AP axis is determined by the position of the polar follicle cells. The inductive signal from the oocyte then imposes polarity on this axis by directing the cells at one end of the egg chamber to adopt a posterior fate.

Because the AP and DV axes are independent later in oogenesis and during early embryogenesis²⁰, the relative orientation of the two axes is determined by the positions of the posterior and dorsal follicle cells. These depend on the placement of the germinal vesicle, and the concomitant localization of *grk* mRNA, first to the posterior of the oocyte and then to the dorsal anterior corner. Our results indicate that the two axes are not independent at this stage, as the polarization of the oocyte microtubule cytoskeleton along the AP axis directs the movement of the germinal vesicle to the anterior of the oocyte (Fig. 6). Thus AP is the primary axis in *Drosophila*, and DV the secondary. Indeed, it is the anterior movement of the germinal vesicle and *grk* mRNA that is responsible for the orthogonal orientation of the two axes.

It is hard to imagine how two perpendicular axes can be defined unless the orientation of one depends on the other. In this context, it is interesting to note that there is evidence that DV axis formation in *Xenopus* also depends on the prior establishment of the primary axis. The animal–vegetal axis forms during oogenesis, leading to the localization of the germ plasm and several maternal mRNAs at the vegetal pole of the oocyte⁴¹. In the freshly laid egg, the vegetal cytoplasm contains an unidentified, but transplantable, dorsalizing activity⁴². After fertilization, a microtubule-dependent process associated with cortical rotation moves this activity from the vegetal pole of the egg to a site in the equatorial region, where it is thought to induce DV polarity^{42,43}. Thus it may be that, in *Xenopus* as in *Drosophila*, a dorsalizing signal moves from the site of germ plasm formation to the future dorsal side, thereby defining a second axis that is perpendicular to the first. □

Received 12 May; accepted 30 May 1995.

1. Spradling, A. in *The Development of Drosophila melanogaster* (eds Bate, M. & Martínez-Arias, A.) 1–70 (Cold Spring Harbor Laboratory Press, New York, 1993).
2. González-Reyes, A. & St Johnston, D. *Science* **266**, 639–642 (1994).
3. Ruohola, H. et al. *Cell* **66**, 433–449 (1991).
4. Lane, M. & Kalderon, D. *Genes Dev.* **8**, 2986–2995 (1994).
5. Theurkauf, W., Smiley, S., Wong, M. & Alberts, B. *Development* **115**, 923–936 (1992).
6. Clark, I., Giniger, E., Ruohola-Baker, H., Jan, L. & Jan, Y. *Curr. Biol.* **4**, 289–300 (1994).
7. St Johnston, D., Driever, W., Berleth, T., Richstein, S. & Nüsslein-Volhard, C. *Development* (Suppl.) **107**, 13–19 (1989).
8. Pokrywka, N. J. & Stephenson, E. C. *Development* **113**, 55–66 (1991).
9. Ephrussi, A., Dickinson, L. K. & Lehmann, R. *Cell* **66**, 37–50 (1991).
10. Kim-Ha, J., Smith, J. L. & Macdonald, P. M. *Cell* **88**, 23–35 (1997).
11. Driever, W. in *The Development of Drosophila melanogaster* (eds Bate, M. & Martínez-Arias, A.) 301–324 (Cold Spring Harbor Laboratory Press, New York, 1993).
12. Ephrussi, A. & Lehmann, R. *Nature* **358**, 387–392 (1992).
13. Schüpbach, T. *Cell* **49**, 699–707 (1987).
14. Neuman-Silberberg, F. & Schüpbach, T. *Cell* **75**, 165–174 (1993).
15. Price, J., Clifford, R. & Schüpbach, T. *Cell* **56**, 1085–1092 (1989).
16. Schejter, E. & Shilo, B.-Z. *Cell* **56**, 1093–1104 (1989).
17. Schüpbach, T. & Wieschaus, E. *Genetics* **129**, 1119–1136 (1991).
18. Brand, A. & Perrimon, N. *Genes Dev.* **8**, 629–639 (1994).
19. Chasan, R. & Anderson, K. in *The Development of Drosophila melanogaster* (eds Bate, M. & Martínez-Arias, A.) 387–424 (Cold Spring Harbor Laboratory Press, New York, 1993).
20. Roth, S. & Schüpbach, T. *Development* **120**, 2245–2257 (1994).
21. Montell, D., Rorth, P. & Spradling, A. *Cell* **71**, 51–62 (1992).
22. Fasano, L. & Kerridge, S. *Development* **104**, 245–253 (1988).
23. Tolias, P. & Kafatos, F. *EMBO J* **9**, 1457–1464 (1990).
24. Cheung, H.-K., Serano, T. & Cohen, R. *Development* **114**, 653–661 (1992).
25. St Johnston, D., Beuchle, D. & Nüsslein-Volhard, C. *Cell* **66**, 51–63 (1991).
26. Giniger, E., Wells, W., Jan, L. & Jan, Y. *Wilhelm Roux Arch. devl Biol.* **202**, 112–122 (1993).
27. Kammermeyer, K. & Wadsworth, S. *Development* **100**, 201–210 (1987).
28. Shilo, B. & Raz, E. *Trends Genet.* **7**, 388–392 (1991).
29. Frohnhofer, H. Thesis, Univ. Tübingen (1982).
30. Brunner, D. et al. *Cell* **76**, 875–888 (1994).
31. Marshall, C. *Cell* **80**, 179–185 (1995).
32. Manseau, L. J. & Schüpbach, T. *Genes Dev.* **3**, 1437–1452 (1989).
33. Lantz, V., Chang, J., Horabin, J., Bopp, D. & Schedl, P. *Genes Dev.* **8**, 598–613 (1994).
34. Christensen, L. & McKearin, D. *Genes Dev.* **8**, 614–628 (1994).
35. Wieschaus, E., Marsh, J. & Gehring, W. *Wilhelm Roux Arch. devl Biol.* **184**, 75–82 (1978).
36. Ashburner, M. et al. *Genetics* **126**, 679–694 (1990).
37. Schüpbach, T., Clifford, R., Manseau, L. & Price, J. in *Cell-cell Interactions in Early Development* (ed. Gerhart, J.) 163–174 (Wiley-Liss, New York, 1991).
38. Koch, E. & Spitzer, R. *Cell Tissue Res.* **228**, 21–32 (1983).
39. Hou, X., Chou, T.-B., Melnick, M. & Perrimon, N. *Cell* **81**, 63–71 (1995).
40. Grossniklaus, U., Bellen, H., Wilson, C. & Gehring, W. *Development* **107**, 189–200 (1989).
41. Klymkowsky, M. W. & Karnovsky, A. *Devl Biol.* **165**, 372–384 (1994).
42. Fujisue, M., Kobayakawa, Y. & Yamana, K. *Development* **118**, 163–170 (1993).
43. Yuge, M., Kobayakawa, Y., Fujisue, M. & Yamana, K. *Development* **110**, 1051–1056 (1990).

ACKNOWLEDGEMENTS. We thank I. Clark, M. Domínguez, E. Hafen, S. Kerridge, D. McKearin, D. Montell, F. Neuman-Silberberg, C. Nüsslein-Volhard, T. Schüpbach and P. Tolias for fly stocks and plasmids; P. Lawrence for advice on pole cell transplants, and J. Casanova, S. Grüner, M. Martín-Bermudo, D. Micklem, M. Weston and C. Yamada for comments on the manuscript. A.G.-R. was a European Molecular Biology Organisation postdoctoral fellow. This work was supported by a Wellcome Trust Senior Fellowship (D.S.U.) and a European Community Postdoctoral Fellowship (A.G.-R.).