Gurken meets *torpedo* for the first time

Intercellular communication between oocyte and follicle cells, mediated by the *gurken–torpedo/DER* signalling pathway, has a crucial role in determining both anterior–posterior and dorsal–ventral polarity in *Drosophila*.

The development of embryonic polarity in the fruitfly *Drosophila melanogaster* requires the formation of gradients of determinant molecules [1]. Opposing gradients of Bicoid (Bcd) and Nanos (Nos) proteins emanate from the embryonic poles and determine polarity along the anterior–posterior axis through activation of genes differentially in nuclei occupying different positions along this axis. Similarly, a graded distribution of Dorsal protein within nuclei around the circumference of the embryo directs dorsal–ventral polarity. The generation of these molecular asymmetries after fertilization relies on the prior establishment of polarized axes during oogenesis by signalling events between the oocyte and the surrounding somatic follicle cells. Results reported in two recent papers [2,3] show that the same signalling pathway specifies both anterior–posterior and dorsal–ventral axes, and that, contrary to what was previously thought, the two axes are not independent of one another.

The *Drosophila* oocyte develops within an egg chamber consisting of an oocyte and its 15 sister nurse cells, which derive from the divisions of a single germline stem cell, and a surrounding epithelium of somatic follicle cells [4]. Anterior–posterior asymmetry becomes evident before dorsal–ventral asymmetry, with the movement of the oocyte to one end of the egg chamber (the future posterior end) early in oogenesis. A signal produced by the oocyte consequently reaches only the adjacent follicle cells at that end of the egg chamber. A specialized subset of these follicle cells, the polar cells, respond to the signal from the oocyte and differentiate with posterior fates. The posterior polar cells then signal back to the oocyte to induce polarization of the oocyte's anterior–posterior axis.

These intercellular communication events were uncovered by analyses of mutations in several genes that affect both follicle-cell identity and oocyte polarity These are 'maternal-effect' mutations, the phenotypes of which are manifested in oocytes produced by homozygous female flies. Mutations in the *spindle-C* (*spn-C*) gene disrupt the movement of the oocyte to the posterior end of the egg chamber [5]. As a result, posterior polar cells of *spn-C* mutants no longer receive the signal from the oocyte, and adopt default anterior fates. Mutations in *Notch (N)* and *Delta (Dl)* [6] produce an excess of polar follicle cells at the posterior end, which may interfere with reception or processing of the oocyte signal. Disruption of patterning of the follicle-cell epithelium in *spn-C*, *N* and *Dl* mutants results in the mislocalization of determinant mRNAs within the oocyte [5,6]. The *bcd* RNA that is normally localized to the anterior end becomes distributed to both poles of oocytes from these mutants, and *oskar (osk)* RNA — whose posterior localization in wild-type oocytes determines the site of pole-plasm formation and thus *nos* RNA localization [7] — accumulates aberrantly in the center. Proper localization of *bcd* and *osk* RNAs in the oocyte is essential for production of the Bcd and Nos protein gradients in the embryo [7]. As the function of *spn-C* is required in the germline [5], whereas *N* and *Dl* act in the follicle cells [6], the similarity of their mutant defects in *bcd* and *osk* RNA localization suggests that reciprocal signalling events between the oocyte and the follicle cells induce the polarized localization of these RNAs that determines the anterior–posterior pattern of the embryo.

The ability of the posterior polar cells to direct RNA localization appears to require a signal from these cells to the oocyte that induces reorganization of the oocyte's microtubule cytoskeleton. Early in oogenesis, an organizing center at the posterior pole of the oocyte extends 'plus' ends of microtubules towards the anterior pole. During mid-oogenesis, when *bcd* and *osk* RNAs become localized, this posterior organizing center disappears and a gradient of microtubules emanates from the anterior cortex, with plus ends oriented towards the posterior pole [8]. Appropriately, a kinesin–β-galactosidase fusion protein (kin–β-gal) that serves as a marker for microtubule polarity is localized to the posterior pole in mid-oogenesis [9]. The kin–β-gal fusion protein, like *osk* RNA, is mislocalized to a central region of oocytes from *spn-C*, *N* and *Dl* mutants [5,6], indicating a symmetrical organization of the microtubule cytoskeleton, with the plus ends oriented towards the oocyte center. As localization of both *bcd* and *osk* RNAs is microtubule-dependent [9,10], mislocalization of *bcd* and *osk* RNAs in oocytes from *spn-C*, *N* and *Dl* mutants likely results from the defect in microtubule reorganization.

Although these studies revealed the role of communication between the oocyte and follicle cells in establishing anterior–posterior polarity, the underlying signalling pathways remained unidentified. Extensive analysis of dorsal–ventral patterning, however, had previously shown its dependence on oocyte–follicle-cell communication and identified the key signalling molecules involved [11]. Dorsal–ventral polarity can first be detected during mid-oogenesis, with the movement of the oocyte nucleus to an anterior corner of the oocyte. A signal produced by the *gurken (grk)* gene, which shows similarity to the transforming growth factor α (TGFα) family of growth

factors, is transmitted locally from the oocyte to the follicle cells that overly this region. Reception of this signal by the follicle cells requires the product of the *torpedo/DER* (*top/DER*) gene, which encodes the *Drosophila* homolog of the epidermal growth factor receptor. Consequently, follicle cells receiving the *grk* signal differentiate with dorsal fates, while the remainder adopt ventral fates. By a poorly understood mechanism, dorsal–ventral differentiation of the follicle cells leads to the ventral activation of a second pathway, which transmits a signal to the fertilized embryo to generate the gradient of nuclear Dorsal protein.

Striking results reported recently by González-Reyes *et al.* [2] and Roth *et al.* [3] show that the *grk–top/DER* signalling pathway also provides the communication from the oocyte to the polar follicle cells that is crucial for the establishment of anterior–posterior polarity (Fig. 1). Careful examination of the mutant phenotypes of *grk*, *top/DER* and *cornichon* (*cni*) — another gene involved in *grk–top/DER* signalling — revealed defects in anterior–posterior patterning [2,3] in addition to the previously described defects in dorsal–ventral patterning. In egg chambers from flies homozygous for strong *grk* alleles, all polar follicle cells adopt anterior fates, as seen by expression of the anterior follicle-cell-specific marker *slow border cells* (*slbo*) and production of the anterior-specific micropyle structure at both ends. As in dorsal–ventral patterning, the requirement for *grk* in determining posterior polar follicle cell fate is germline-dependent, indicating that *grk* produces the signal sent by the oocyte to the posterior polar cells. Consequently, the investigators tested the requirement for *top/DER* in this process [2,3].

As *top/DER* acts in a number of signalling processes throughout development, complete loss-of-function mutations are lethal. For this reason, less severe *top/DER* alleles that allow homozygous females to survive and produce oocytes were examined. In addition to the previously described dorsal–ventral defects, a significant proportion of egg chambers from such hypomorphic *top/DER* mutants show duplication of *slbo*-expressing cells and micropyle formation at the posterior end of the oocyte. As *top* function shows no germline requirement, *top/DER* must be acting in the posterior follicle cells, presumably for reception of the signal from the oocyte. Mutations in *cni* produce defects identical to those observed for *grk* and *top* in anterior–posterior and dorsal–ventral patterning, providing further evidence that the same signalling pathway induces polarization of both axes [2,3]. The *cni* gene encodes a novel, hydrophobic protein that, like Grk protein, is required in the germline and may be involved in producing an active *grk* signal [3].

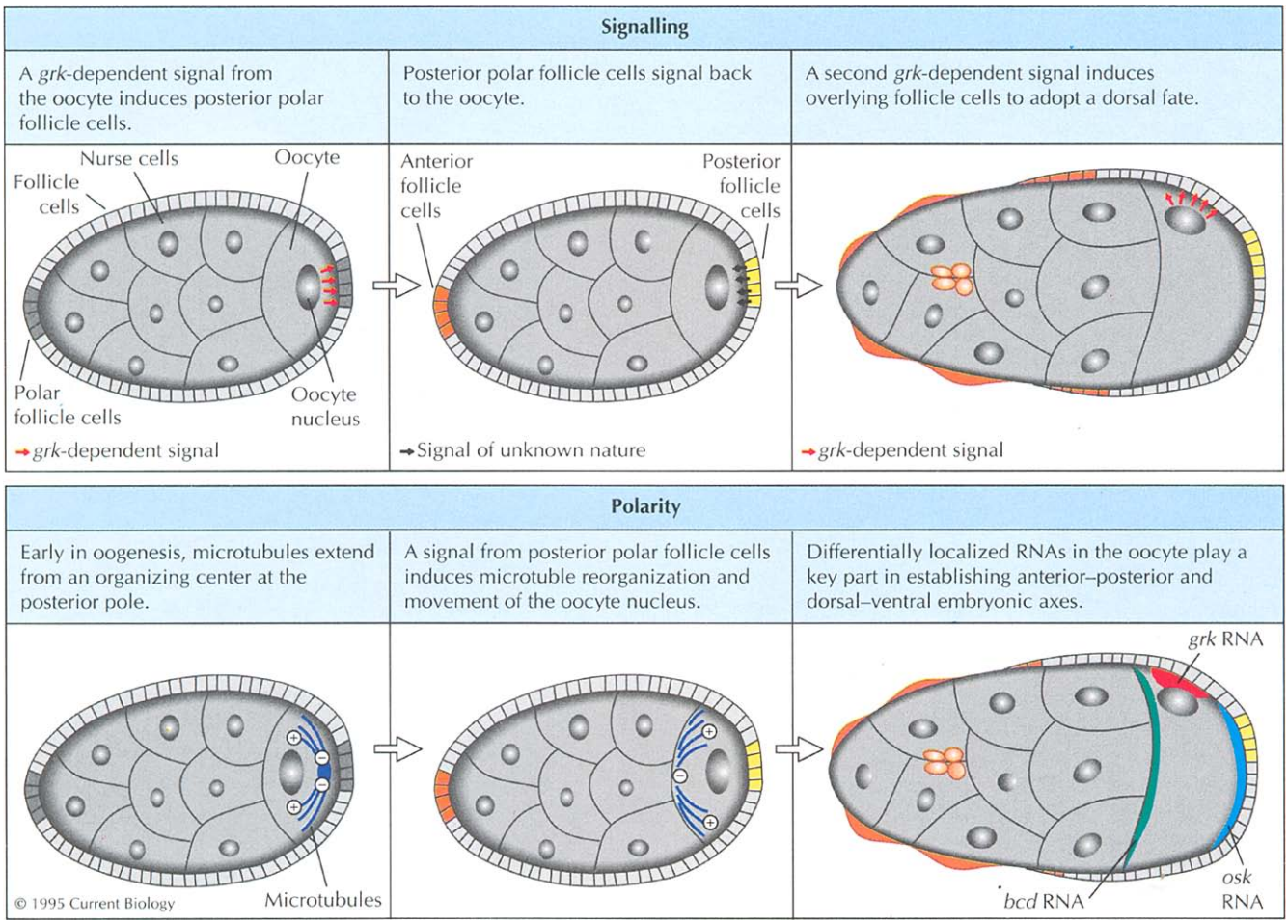

Fig. 1. Determination of anterior–posterior and dorsal–ventral polarity by the *grk–top* signalling pathway.

If the *grk–top/DER* pathway mediates signalling from the oocyte to induce posterior follicle cell fates, then mutations in *grk*, *top/DER* and *cni* would be predicted to disrupt anterior–posterior polarization of the oocyte. Indeed, *bcd* and *osk* RNAs and the kin–β-gal fusion protein are mislocalized in oocytes from *grk*, *top/DER* and *cni* mutants [2,3], just as they are in oocytes from *spn-C*, *N* and *Dl* mutants [5,6]. Females homozygous for strong *grk*, *top/DER* and *cni* mutant alleles do not produce fertilized embryos, but the few developing embryos produced by females homozygous for weaker *grk* and *cni* mutant alleles show defects in anterior–posterior patterning [3]. Thus, the failure of signalling between the oocyte and polar follicle cells, whether because of a defect in the distribution (*spn-C*), production (*grk*, *cni*) or reception (*N*, *Dl*, *top/DER*) of the signal, leads to failure to reorganize the microtubule cytoskeleton, consequent loss of polarized RNA localization and a failure in patterning of the anterior–posterior axis.

Top/DER protein is present throughout the follicular epithelium. Spatial restriction of the *grk* signal ensures the localized activation of Top/DER [12]. In early egg chambers, the position of the oocyte limits the distribution of the Grk protein to the region of the future posterior follicle cells. Later, during mid-oogenesis, localization of *grk* RNA to an anterior corner of the oocyte, in close association with the oocyte nucleus, provides a critical means of restricting Grk protein to the future antero-dorsal side. A key finding reported by González-Reyes *et al.* [2] and Roth *et al.* [3] is that the movement of the oocyte nucleus and restriction of *grk* RNA to an anterior corner during mid-oogenesis is often disrupted in oocytes from *grk*, *top/DER* and *cni* mutants. The dorsal–ventral polarity defects of other mutants that disrupt *grk* RNA localization but not movement of the nucleus indicate that *grk* RNA localization is essential both to providing and restricting signalling by Grk to the dorsal follicle cells during this second event [11,12]. As movement of the nucleus is microtubule-dependent [13], the repolarization of the microtubule cytoskeleton induced by the posterior polar follicle cells appears to drive this anterior migration and thus to put the dorsal signalling mechanism into position.

Although anterior–posterior and dorsal–ventral polarity had previously been thought to be generated by largely independent processes, these new results show clearly that, during oogenesis, dorsal–ventral polarization requires prior establishment of anterior–posterior polarity. Proper specification of posterior follicle cell fate leads to reorganization of the oocyte's microtubule cytoskeleton, directing the localization of *bcd*, *osk* and *grk* RNAs that will determine embryonic polarity. The demonstration that the *grk–top/DER* signalling pathway acts twice during oogenesis, once to determine the fate of the posterior polar follicle cells and once to determine dorsal follicle fate, further links anterior–posterior and dorsal–ventral axis formation.

The ability of the same signalling pathway to induce different follicle-cell fates is likely to rely on the prior specification of a subset of follicle cells as polar, allowing them to respond to the signal differently from the future dorsal follicle cells, but the mechanism underlying this initial differentiation is unknown. Another important unresolved question concerns the nature of the signal from the posterior polar cells to the oocyte that induces reorganization of the oocyte cytoskeleton. One candidate for an effector in this pathway is protein kinase A, whose activity is required in the germline and whose mutant phenotype includes defects in microtubule reorganization and mislocalization of *bcd* and *osk* RNAs [14]. Phosphorylation by protein kinase A may drive the reorganization, but the details of this pathway and its input from the follicle cells remain to be elucidated.

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