INTRODUCTION

Patterning the follicle cells that cover the developing oocyte determines the polarity of the *Drosophila* embryo. This is achieved by an elaborate communication between the oocyte and the surrounding follicle cells. Eventually, signals from the follicle cells determine the polarity of the future embryonic axes. The specification of at least three subsets of follicle cells, posterior, dorsal and terminal, is responsible for determining the anterioposterior and dorsoventral axes of the embryo, and the terminal structures, respectively (reviewed by Ray and Schüpbach, 1996). Surprisingly, the pathway triggered by the *Drosophila* EGF receptor (DER/Torpedo/EGFR) is responsible for the determination of both posterior and dorsal follicle cell fates, at two discrete stages of oogenesis (González-Reyes et al., 1995; Roth et al., 1995; Price et al., 1989).

The anterioposterior axis is the first to be specified in the developing oocyte. From the initial stages of oogenesis, the population of follicle cells covering the oocyte is not uniform. Situated at both termini is a small group of polar follicle cells that has been determined at an earlier stage (Ruohola et al., 1991; Margolis and Spradling, 1995). There appears to be no distinction at this phase however, between the anterior and posterior follicle cells. Prior to stage 7, *gurken* transcripts, encoding a TGFβ homologue that is likely to be a ligand for DER, are localized at the space between the oocyte nucleaus and the posterior part of the oocyte (Neuman-Silberberg and Schüpbach, 1993). DER, in contrast, is expressed in all follicle cells (described below). Activation of DER by Gurken at the posterior follicle cells induces posterior follicle cell fates, which are distinct from the default fate that is maintained in the anterior follicle cells. The posterior cells then signal back to the oocyte, through an unknown mechanism. Consequently, reorganization of the microtubule and microfilament system of the oocyte ensues, and the localization of mRNAs (e.g. *bicoid* and *oskar*) along the anterioposterior axis takes place. This system is also responsible for directing migration of the oocyte nucleus to an anterior peripheral position in the oocyte, which will subsequently become the dorsal region.

Migration of the oocyte nucleus is completed by stage 8-9. In parallel to the continuous growth of the oocyte, posterior migration of follicle cells over the oocyte is observed. By stage 10 the nurse cells, which occupy half of the egg chamber, are covered by only approx. 50 thin follicle cells (the stretch cells), while the oocyte itself is surrounded by the remaining approx. 1,000 follicle cells, which are columnar in shape (reviewed by Spradling, 1993).

*gurken* transcripts maintain their close association with the migrating oocyte nucleus (Neuman-Silberberg and Schüpbach, 1993). After completion of nuclear migration, a second phase of DER activation takes place, this time leading to the induction of dorsal follicle cell fates. The dorsal-anterior cells

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**SUMMARY**

Previous work has demonstrated a role for the *Drosophila* EGF receptor (Torpedo/DER) and its ligand, Gurken, in the determination of anterioposterior and dorsoventral axes of the follicle cells and oocyte. The roles of DER in establishing the polarity of the follicle cells were examined further, by following the expression of DER-target genes. One class of genes (e.g. *kekon*) is induced by the DER pathway at all stages. Broad expression of *kekon* at the stage in which the follicle cells migrate posteriorly over the oocyte, demonstrates the capacity of the pathway to pattern all follicle cells except the ventral-most rows. This may provide the spatial coordinates for the ventral-most follicle cell fates. A second group of target genes (e.g. *rhomboid*) is induced only at later stages of oogenesis, and may require additional inputs by signals emanating from the anterior, stretch follicle cells. The function of Rho was analyzed by ectopic expression in the stretch follicle cells, and shown to induce a non-autonomous dorsalizing activity that is independent of Gurken. Rho thus appears to be involved in processing a DER ligand in the follicle cells, to pattern the egg chamber and allow persistent activation of the DER pathway during formation of the dorsal appendages.

Key words: EGF receptor, Oogenesis, Rhomboid, Gurken, *Drosophila*
express rhomboid (rho) (Ruohola-Baker et al., 1993), which was shown to be an integral member of the DER signaling cassette in other tissues (Sturtevant et al., 1993; Noll et al., 1994; Schweitzer et al., 1995; Golembo et al., 1996; Gabay et al., 1997). The presence of Rho in these cells appears essential for normal patterning of the follicle cells and embryo (Ruohola-Baker et al., 1993). Ventral follicle cells which do not receive DER-induced signaling will assume the default, ventral cell fate. The differentiated ventral follicle cells generate a ventralizing signal that is mediated by the products of the pipe, nudel and wbl genes. These genes are required at early stages of embryogenesis, for restricting the domain in which Spätzle will be proteolytically processed, to produce an active ligand of Toll (reviewed in Chasan and Anderson, 1993; Ray and Schüpbach, 1996).

Several issues regarding patterning of the dorsoventral axis in the oocyte remain open. The mechanism by which activation of the DER pathway in the dorsal follicle cells leads to the correct patterning of the ventral follicle cells and subsequently of the embryo, is not clear. In particular, it is difficult to understand how a region of high DER activity in the dorsal-anterior patch of follicle cells is capable of inducing a putative stripe of ventral follicle cells running along the entire length of the egg chamber.

In addition, the mechanistic basis for the function of Rho in the ovary is not clear. rho encodes a protein with multiple putative transmembrane domains (Bier et al., 1990). Several lines of evidence suggest that in the embryo Rho may participate in the processing of the transmembrane form of Spitz to generate a secreted, active ligand. Rho was shown to function non-autonomously, e.g. expression of Rho only in the midline of the oocyte remains open. The mechanism by which activation of the DER pathway in the dorsal follicle cells leads to the correct patterning of the ventral follicle cells and subsequently of the embryo, is not clear. In particular, it is difficult to understand how a region of high DER activity in the dorsal-anterior patch of follicle cells is capable of inducing a putative stripe of ventral follicle cells running along the entire length of the egg chamber.

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In this work we dissect the roles of the DER signaling pathway at large, and Rho in particular, during oogenesis. We show that immediately after the movement of the oocyte nucleus to the future dorsal pole, during the posterior migration of the follicle cells, a broad activation of the DER pathway takes place. As a result, all follicle cells, except the ventral-most rows, express DER-target genes. This stage may be responsible for the establishment of follicle cell fate, as the default state. After completion of cell migration, transcription of rho in the dorsal-anterior follicle cells is achieved by activation of the DER pathway, in conjunction with signals that may emanate from the anterior, stretch follicle cells. Like its embryonic functions, the role of Rho in the ovary is non-autonomous. Ectopic expression of Rho in the stretch follicle cells, positioned around the nurse cells, can lead to activation of the DER pathway in the follicle cells covering the oocyte. These results suggest that Rho is responsible for triggering the production or processing of a DER ligand that is expressed in the follicle cells. Mutant follicle cell clones for rho, Star and spitz, as well as genetic interactions, confirm the requirement for these genes in the follicle cells, to form and pattern the egg chamber.

MATERIALS AND METHODS

Fly lines

The following lines were used: 55B-Gal4 (obtained from A. Brand), AN296 (obtained from T. Schüpbach), AA69 (obtained from S. Crews), 2.2rho (obtained from M. Levine), HS-ras* (obtained from N. Perrimon), UAS-rho 24-2 (on the 2nd chromosome) and UAS-spitz 4a, grkHrk5, grkWGo1, top1, top2 and fs(1)K10 were obtained from T. Schüpbach. For a homozygous grk or top background, females carrying two different alleles were generated. The following mutations in the spitz group genes were used: spzOE92 (obtained from N. Perrimon), spzIT23, spzIS14, snl22s (obtained from C. Nüsslein-Volhard), Df(2R)LS-3 (removing the S locus). We also used argosA7 (obtained from M. Freeman) and SosA6c (obtained from M. Simon). For follicle cell clones fs(2)Ugra and fs(3)Apc were used (obtained from J. Szabad).

Ovary staining and antibodies

X-Gal staining was according to Margolis and Spradling (1995). Fixation of ovaries was in 0.5% glutaraldehyde. Antibody staining was according to Hsu et al. (1996), and fixation of ovaries was in 4% paraformaldehyde. The following antibodies were used: mouse polyclonal anti-DER was generated against the C-terminal EcoRI fragment inserted into pRSET, and rabbit anti-β-Gal (Cappel). Specificity of anti-DER antibodies was verified by the expected embryonic expression pattern, and by absence of staining in embryos homozygous for a deficiency uncovering the DER locus. To visualize nuclei, egg chambers were treated with RNaseH (400 μg/ml) for 15 minutes after staining, washed and incubated with Oli green (Molecular Probes 1/500). Secondary antibodies were purchased from Jackson laboratories. Chorion morphology was monitored by mounting in Hoyers, and embryonic cuticles were visualized following dechorionation and devitellinization, after mounting in Hoyers/vlactic acid 1:1.

Induction of constructs and mosaic clones

For all Gal4/UAS inductions, flies were grown at 18°C until hatching, and transferred to 25°C. Heat-shock based constructs were induced twice at 37°C for 20-30 minutes in a water bath, with a 2-hour interval. Four hours after the second heat shock, ovaries were dissected for staining, and 15-30 hours after heat shock eggs were collected.

For generation of mosaic follicle cell clones, males carrying fs(2)Ugra or fs(3)Apc were crossed to females carrying the mutation of interest. Second to third instar progeny were X-irradiated with 1,500 Rad. Hatched females (at least 15) carrying the dominant female sterile allele over the mutation of interest were collected and their capacity to lay eggs was analyzed.

RESULTS

DER expression in the ovary

Pole cell transplantation experiments have demonstrated that DER/Torpedo is required in the follicle cells but not in the germ line, in contrast to Gurken which is required only in the germ line (Schüpbach, 1987). In view of the diverse roles of DER in oogenesis, it was important to determine its expression pattern. DER antibodies were used to stain ovaries. Membranal staining, that is restricted to the follicle cells was detected. It
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...initiates in the germarium in the middle of region 2, where inwardly migrating follicle cells cover the 16-cell germ line cyst (Fig. 1A). DER continues to be expressed uniformly in the follicle cells when individual egg chambers are formed at stage 2 of oogenesis (Fig. 1A), until stage 10 (Fig. 1B-E). Most egg chambers displayed uniform staining in all follicle cells at stage 10 (Fig. 1C,D). In some egg chambers however, a clear reduction in the level of DER was detected, specifically in the dorsal-anterior follicle cells above the oocyte nucleus (Fig. 1E). This reduction appears to result from decreased transcription of DER, as a similar pattern is seen by DER RNA in situ hybridization (not shown). Reduc...
significantly reduced, but at stage 9/10, only a dorsal stripe of expression is observed, in the follicle cells that were immediately over the oocyte nucleus during the posterior migration of these cells (Fig. 2G,H). Since grk and top may not represent null alleles, the remaining expression of AN296 correlates with the site of maximal gurken concentration, and therefore with the highest residual DER activation. Taken together, these observations suggest that activation of the DER signaling pathway is necessary and sufficient to induce AN296 transcription.

Expression of Rhomboid in the ovary

Rho expression in the ovary begins only at stage 10, as a triangle of follicle cells positioned directly above the oocyte nucleus (Ruohola-Baker et al., 1993), and is also observed in the centripetal follicle cells positioned between the oocyte and nurse cells. Subsequently, rho expression in the dorsal-most cells becomes confined to two smaller triangles at stage 10-11 (Neuman-Silberberg and Schüpbach, 1994). This group of cells will give rise to the two dorsal appendages. Anterior migration of these cells over the growing end, and subsequent secretion of chorion gives rise to the typical structure of the dorsal appendages. An enhancer trap in the rho gene (termed AA69) faithfully represents the reported pattern of the rho transcripts. It is first expressed in the triangle over the oocyte nucleus, and subsequently in the migrating dorsal appendage cells (Fig. 3A,D). A rho promoter reporter (2.2rho) is initially expressed at stage 10B in a subset of follicle cells that will give rise to the dorsal appendages, and continues to be expressed in these cells at subsequent stages (Fig. 3B,C).

The DER pathway participates in the initial induction of rho. In egg chambers derived from females containing multiple
copies of the normal grk gene, an expanded expression of rho was monitored (Neuman-Silberberg and Schüpbach, 1994). In fs(1)K10 mutant egg chambers, or following ectopic expression of activated Ras in the follicle cells, an expansion of rho expression forming an anterior ring is observed (Fig. 3E,F). Finally, in grk or top mutant egg chambers, rho is not expressed in the dorsal follicle cells. Normal expression is retained, however, in the centripetal follicle cells, positioned between the oocyte and nurse cells (Ruohola-Baker et al., 1993; Fig. 3G,H).

Non-autonomous activity of Rho in the ovary
Expression of rho in the dorsal-anterior follicle cells is essential for dorsoventral patterning by DER (Ruohola-Baker et al., 1993). Since DER and Rho are expressed in the follicle cells and the ligand Gurken in the oocyte, it was inferred that the activity of Rho is required for cells expressing the receptor to receive the signal. However, we have recently proposed that Rho is involved in posttranslational processing of the DER ligand Spitz (Schweitzer et al., 1995; Golembo et al., 1996). To check if this is also true in the egg chamber, it was important to see if in this system Rho can also exert a non-autonomous effect. To address this issue, we wanted to express Rho in cells that do not participate in dorsoventral patterning, such that resulting dorsoventral defects will clearly be attributed to a non-autonomous activity.

The group of ~50 stretch follicle cells do not normally experience the activation of DER, since they are never in direct contact with the oocyte, which is the source for Gurken. To induce Rho expression in the stretch cells, the 55B Gal4 inducer line expressing Gal4 exclusively in these cells was used. Gal4 is first expressed at stage 7 in the anterior follicle cells. Expression continues during stages 9-10, as these cells extend over the nurse cells. By stage 10, Gal4 is expressed in the follicle cells covering all nurse cells and in the centripetal cells, but is not expressed in the follicle cells positioned above the oocyte, or in the nurse cells (Fig. 4).

Expression of secreted Spitz in the stretch follicle cells induced by the 55B line, gave rise to non-autonomous effects, as monitored by the appearance of dorsal appendages throughout the anterior circumference of the egg (Fig. 5B). A small fraction of eggs was fertilized, and developed into dorsalized embryos (not shown). Thus, a secreted DER ligand produced only in the stretch follicle cells, has the capacity to diffuse and pattern the follicle cells covering the oocyte. The system was then used to express rho ectopically in the stretch follicle cells, and the results were very similar to the ones monitored following expression of secreted Spitz (Fig. 5C). The observed effects following ectopic expression of Rho in the stretch cells, which are not in contact with the oocyte,
suggested that Grk is not involved in the process. To address this issue directly, the same induction of rho was monitored in grk mutant egg chambers. In a grk mutant background, expression of the 55B-Gal4 line which is typically restricted to the anterior follicle cells, is also extended to patches of posterior follicle cells (Fig. 4D). This may be explained by the failure to induce posterior follicle cell fates and the retention of a default anterior fate in a grk mutant background (González-Reyes et al., 1995; Roth et al., 1995; Twombly et al., 1996). Induction of Rho by 55B-Gal4 in grk females resulted in deposition of dorsal appendage material around the anterior circumference of the egg chamber (Fig. 5E). In addition, dorsal appendage material was also deposited at the posterior end of the egg chamber, according to the expression pattern of the 55B inducer line. This indicates that the effects of ectopic Rho are not mediated by Grk.

The consequences of ectopic rho expression in the stretch cells were further monitored with molecular markers. The effect on the expression of AN296 was subtle, as no pronounced expansion of expression in the follicle cells over the oocyte was observed. However, induction of the marker could be detected in several of the stretch cells (Fig. 5F). This is consistent with the expression of DER in these cells (Fig. 1D). Expression of the rho AA69 marker provided a more compelling indication for the non-autonomous effects of Rho expression in the stretch cells. Expansion of the marker in a ring-like structure surrounding the anterior part of the egg chamber was detected (Fig. 5G). In grk mutant egg chambers, a similar anterior ring of AA69 expression was induced by ectopic Rho (Fig. 5H). We found again that under these conditions, additional follicle cells also express the marker (see Discussion).

**Requirement of spitz group genes for Rhomboid effects**

While many of the egg chambers formed following ectopic Rho expression could not be fertilized due to excess dorsal appendage material around the micropyle, some fertilized eggs were obtained. Cuticle preparation of these embryos revealed that they were dorsalized, as can be inferred from the absence or almost complete elimination of denticle bands, and the expansion of dorsal hairs (Fig. 6A). This result indicates that ectopic expression of Rho in the stretch cells has the capacity not only to alter the pattern of marker gene expression in the follicle cells, but also to interfere with dorsoventral patterning of these cells and of the embryo.

Apart from rho, the sites of expression in oogenesis and possible roles for other genes in the DER signaling cassette are not known. The effects of ectopic Rho expression were used to provide a sensitized genetic background, to determine the involvement of other members of the DER signaling pathway. Heterozygosity for the Star gene was previously reported to suppress the phenotypic effects of ectopic rho in many tissues, including the egg chamber (Noll et al., 1994). However, these experiments did not indicate whether Star is required during the activation of DER by Gurken, or at subsequent stages. In females heterozygous for a Star mutation, dramatic suppression of the ectopic rho phenotype is observed. The dorsal appendages appeared more normal, and the embryo displayed reduced but distinct ventral denticle bands (Fig. 6B). To test if Spitz may participate in the Rho-induced effects, the phenotype was monitored in embryos laid by females heterozygous for a spitz mutation. However, no significant effects on the Rho-induced phenotype were observed in these embryos. These results do not prove that spitz is not involved in the process, since the levels of the Spitz precursor in heterozygous females may be high enough to promote the full biological response.

Genetic interactions were also tested in females heterozygous for gurken. Under these conditions, the level of Grk is halved but not eliminated, and expression of the 55B inducer line remains restricted to the stretch cells. The severity of the phenotype induced by ectopic Rho is reduced. Although some ectopic dorsal appendage material is still produced, the two dorsal appendages are prominent, and the embryos form denticle bands (Fig. 6C). Thus, a lower level of activation by Grk in the heterozygous females, reduced the capacity to form ectopic dorsal appendages. This indicates that the normal activation of DER by Gurken during stages 9 through 10B conditions the follicle cells, and collaborates with ectopic activation by Rho in the formation of dorsal appendages. In the homozygous grk females, the expanded expression of the 55B inducer may compensate for the absence of Grk activity, and allow the extended appearance of dorsal appendages.

**Follicle cell clones of spitz group mutants**

Genetic interactions described above suggested that the Star gene participates in DER signaling in the ovary. The involvement of the different spitz group genes was analyzed by generating follicle cell clones. The method is based on dominant female-sterile mutations termed Ugra and Apc, which map to the left arm of the second and third chromosomes, respectively (Szabad et al., 1989; Szabad and...
requirement for different alleles for each locus gave identical results. The initial recessive female-sterile mutations on the chromosome, since obtaining eggs is not an indirect consequence of additional homozygous mutant clones for these genes. The failure to Sos chromosomes were tested. On 2L, clones for normal eggs, which undergo proper embryonic development. Generation of wild-type clones of follicle cells by X irradiation of heterozygous females at the third instar larval phase, rescues the products are required in the follicle cells and not in the germ line. Hoffman, 1989; Szabad et al., 1991). Pole cell transplantations have demonstrated that the Ugra and Apc gene products are required in the follicle cells and not in the germ line. Females heterozygous for the Ugra mutation arrest the development of the oocyte at stage 10 and do not lay eggs, while females heterozygous for the Apc mutation lay eggs which are abnormal in structure and lack dorsal appendages. Generation of wild-type clones of follicle cells by X irradiation of heterozygous females at the third instar larval phase, rescues the mutant phenotype efficiently: up to 80% of the females lay normal eggs, which undergo proper embryonic development.

Mutations of interest on the arms of the second and third chromosomes were tested. On 2L, clones for spitz, Star and Sos were generated. No eggs were laid following generation of homozygous mutant clones for these genes. The failure to obtain eggs is not an indirect consequence of additional recessive female-sterile mutations on the chromosome, since different alleles for each locus gave identical results. The initial requirement for spitz, Star and Sos may take place at the early stages of oogenesis, as no eggs (rather than eggs with abnormal polarity) were laid. Examination of ovaries of these females did not reveal any egg chambers that were arrested at a particular stage. We cannot conclude whether these genes are also required subsequently, at the phase in which dorsoventral polarity is established. Mutations on the left arm of the third chromosome were also examined. Again, no rescue was observed by follicle cell clones homozygous for rho and argos mutations, no ventralized or dorsalized eggs were laid, and no defective egg chambers were detected in the ovaries. Thus, Rho and Argos may also participate in an early phase of DER signaling.

The inability to rescue the female sterile mutations Ugra and Apc by follicle cell clones homozygous for spitz, Star, Sos and rho mutations demonstrated that these DER pathway genes are required in the follicle cells at the early stages of oogenesis. An early DER function for the initial recruitment and spreading of the follicle cells around the 16-cell germ line cyst was indeed previously described (Goode et al., 1996). Interestingly, a requirement for a second DER ligand in this process had also been suggested, based on the observation that double mutants of brainiac with grk give a phenotype which is less severe than brainiac/torpedo double mutants (Goode et al., 1996). This early requirement for the DER pathway may involve the initial spreading of the follicle cells around the 16-cell germ line cyst. Interestingly, mutations in argos also failed to produce eggs, suggesting that Argos may also be playing a role early in oogenesis as an inhibitory ligand of DER.

**DISCUSSION**

**Sequential activation of the DER pathway in oogenesis**

The EGF receptor is highly pleiotropic, regulating a large number of processes during all stages of Drosophila development (Schweitzer and Shilo, 1997). Oogenesis represents an extreme case, where combined data from previous reports and this work, point to five independent stages in which the DER pathway is activated, all within the monolayer of the follicular epithelium.

By generating follicle cell clones, we have demonstrated that different elements in the DER signaling pathway are essential for the early stages of oogenesis. In the absence of spitz, rho and Star, egg chambers fail to develop. We assume that absence of the DER pathway leads to loss of the integrity of the follicle cell layer covering the germ line cells. The second phase of DER activation takes place prior to stage 7, and is responsible for induction of posterior follicle cell fates triggered by Gurken (González-Reyes et al., 1995; Roth et al., 1995). In the third cycle of DER activation, determination of the dorsoventral axis takes place, after migration of the oocyte nucleus to the future dorsal-anterior corner of the oocyte. While the essential role of the DER pathway in the induction of dorsal cell fates has been widely recognized (Schüpbach, 1987; Price et al., 1989; Brand and Perrimon, 1994; Roth and Schüpbach, 1994b), the precise phase in which this process takes place was not defined.

The expression of AN296 described above, provides a sensitive reporter for DER activation, and clarifies the spatial and temporal aspects of dorsoventral patterning by the pathway. At stages 8-9, when the follicle cells migrate posteriorly over the oocyte, expression of AN296 is very broad. This pattern is induced along the two axes. First, posterior migration of the follicle cells over the source of high Grk induces activation of the DER pathway in all follicle cells passing over the oocyte nucleus. Second, lateral diffusion of the Grk signal leads to a symmetrical lateral activation which decreases towards the ventral follicle cells. Consequently, expression of the marker is induced in all follicle cells, except in the ventral-most rows (Fig. 2A,B). This pattern has the capacity to define, by default, the fate of the ventral-most rows of cells.

The next cycle of DER activation takes place at stage 10, when the follicle cells have completed their posterior migration. A critical event is the induction of rho expression, triggered by Gurken-mediated DER activation. Rho expression is essential for dorsoventral patterning, since expression of antisense rho in all follicle cells can lead to the generation of ventralized egg chambers and embryos (Ruohola-Baker et al., 1993). However, as previously noted, it is implausible that this dorsoanterior expression domain can define the dorsal and ventral regions of the egg chamber. What then is the function of this wave of DER activation with respect to dorsoventral patterning?

One possibility is that this phase has an additive effect to the previous activation of DER, which took place during follicle cell migration. The combined effects of both phases would determine the capacity of the follicle cells to become dorsal. Normally, activation of the DER pathway in the Rho-expressing cells does not seem to extend beyond these cells, as monitored by expression of the DER-target gene kekon. It is thus possible that relay mechanisms extend a second, unknown dorsalizing signal from the Rho-expressing cells to the more lateral and posterior follicle cells.

Finally, Rho-dependent signaling appears to be important for patterning the dorsal appendages. This is supported by the persistent expression of kekon and rho in the precursors of the dorsal appendages until the final stages of oogenesis (Figs 2D, 3D), and by the induction of multiple dorsal appendages following ectopic Rho expression. Patterning of the dorsal appendages may thus represent another distinct DER-dependent process. The stages of DER signaling in the ovary are summarized in Fig. 7.
Dual input for rho expression

Throughout development rho expression prefigures and induces DER activation (Gabay et al., 1997). Induction of rho expression in the ovary by DER is thus unique and represents a positive feedback loop, where DER activity induces rho expression, which in turn will induce DER activation. While the kekon gene is induced at each phase of DER activation, rho expression is triggered only from stage 10 onwards. A mechanism must exist to prevent rho and possibly other genes (e.g. bunched) (Dobens et al., 1997), from being triggered by the same pathway at earlier stages. One option is that induction by the DER pathway is not sufficient to trigger rho, and an additional input provided by a different group of cells, is required. The second input may be a signal emanating from the stretch follicle cells. Dpp is expressed in these cells (Twombly et al., 1996) and may provide a likely candidate. Multiple requirements for triggering rho expression may thus assure that it will normally be induced in a restricted point in space and time, only when the Gurken-induced signal emanating from the oocyte nucleus can be combined with a signal originating from the stretch follicle cells (summarized in Fig. 8).

The requirement for a second input is supported by the experiment in which ubiquitous expression of activated Ras in the follicle cells leads to the induction of rho expression only in an anterior ring of follicle cells, rather than in all follicle cells, is required. The second input may be a signal emanating from the stretch follicle cells. Dpp is expressed in these cells (Twombly et al., 1996) and may provide a likely candidate. Multiple requirements for triggering rho expression may thus assure that it will normally be induced in a restricted point in space and time, only when the Gurken-induced signal emanating from the oocyte nucleus can be combined with a signal originating from the stretch follicle cells (summarized in Fig. 8).

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cells (Fig. 3F). This model may also explain why in a grk mutant background, we observed a broader response to ectopic activation of the DER pathway than in a wild type background. In grk egg chambers, the induction of rho expression was also observed in the posterior and central follicle cells, and the widespread formation of ectopic dorsal appendage material was monitored (Fig. 5E,H). In grk mutant egg chambers, the posterior follicle cells were shown to develop as anterior cells, and express Dpp, among other anterior markers (Twombly et al., 1996). The defective posterior cells may thus provide a second source for the signals necessary to collaborate with DER, leading to expression of rho in all follicle cells. A similar formation of ectopic dorsal appendage material covering all parts of the oocyte was observed when constitutively activated DER was ubiquitously expressed in a grk mutant background (Queenan et al., 1997).

In grk mutants, a small fraction of egg chambers expressed rho in the posterior follicle cells (Fig. 3H). A similar result was obtained for expression of the bunched gene (Dobens et al., 1997). Again, ectopic signals in the posterior follicle cells may occasionally collaborate with residual DER activation in the same cells, to induce expression of rho or bunched.

CF2 is a zinc finger protein shown to function as a repressor of rho transcription in the ovary. Expression of an antisense construct of CF2 resulted in ubiquitous expression of rho in all follicle cells (Hsu et al., 1996). Thus, CF2 degradation or inactivation may represent a stage which integrates the dual input from DER and the additional pathway. The dual requirement for a signal emanating from the stretch cells combined with DER activation, ensures that rho expression will be induced only once the posterior migration of the follicle cells has been completed. This provides a temporal separation between the two phases of DER activation, namely the early Gurken-mediated activation and the subsequent Rho-mediated activation.

The roles of Rho in oogenesis

Two possible mechanisms for Rho activity in the ovary are ruled out by this work. One option was that Rho functions in a cell-autonomous manner to facilitate activation of DER in the follicle cells. The other scenario was that Rho may support cleavage of Gurken which is located on the oocyte membrane. These notions appears less likely now, since Rho was shown to exert its activity in the stretch cells, which are not in contact with the oocyte. Furthermore, the effect of 55B-Gal4/UAS-rho is manifested even in a grk background. We remain with a model for the function of Rho which is based on the corollaries to the embryonic roles of the protein. It is possible that the follicle cells express a precursor for another DER ligand (Spitz or an unknown ligand). Normal expression of rho in the dorsal-anterior cells or ectopic expression in the stretch cells, may allow processing of this putative ligand, followed by its diffusion.

We suggest that this molecule is a ligand for the DER pathway rather than for another receptor system. First, in all other tissues where Rho is expressed during development, it is intimately linked to the activation of the DER pathway (Gabay et al., 1997). Second, ectopic Rho expression in the egg chamber triggers the expression of downstream markers for DER activation, such as kekon and rho itself. Induction of Rho expression in the dorsal follicle cells may allow processing of a ligand expressed in these cells. This should generate a loop of DER activation which is independent of Gurken signal emanating from the oocyte nucleus. It is thus possible to maintain DER signaling in the dorsal appendage cells, even after they migrate from their original position and are no longer in contact with the egg that provides Gurken.

In conclusion, DER signaling emerges as a central pathway for morphogenesis of structural components of the egg chamber such as the follicle cell layer and the dorsal appendages. In addition, it is responsible for establishment of polarity in both anterioposterior and dorsoventral axes. To further understand patterning of the follicle cells by the DER pathway, it will be necessary to decipher how successive activation cycles are integrated in the developmental continuum, and coordinated with other signaling pathways.

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