Merlin, the *Drosophila* homologue of neurofibromatosis-2, is specifically required in posterior follicle cells for axis formation in the oocyte

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**SUMMARY**

In *Drosophila*, the formation of the embryonic axes is initiated by Gurken, a transforming growth factor α signal from the oocyte to the posterior follicle cells, and an unknown polarising signal back to the oocyte. We report that *Drosophila* Merlin is specifically required only within the posterior follicle cells to initiate axis formation. Merlin mutants show defects in nuclear migration and mRNA localisation in the oocyte. Merlin is not required to specify posterior follicle cell identity in response to the Gurken signal from the oocyte, but is required for the unknown polarising signal back to the oocyte. Merlin is also required non-autonomously, only in follicle cells that have received the Gurken signal, to maintain cell polarity and limit proliferation, but is not required in embryos and larvae. These results are consistent with the fact that human Merlin is encoded by the gene for the tumour suppressor neurofibromatosis-2 and is a member of the Ezrin-Radixin-Moesin family of proteins that link actin to transmembrane proteins. We propose that Merlin acts in response to the Gurken signal by apically targeting the signal that initiates axis specification in the oocyte.

Key words: *Drosophila* oogenesis, Merlin, gurken, bicoid, oskar, TGFα, mRNA localisation, Oocyte microtubules, Embryonic axis formation, Tumour suppressor, ERM, Cell signalling

**INTRODUCTION**

The embryonic axes of *Drosophila* are established during oogenesis through the localisation of specific mRNAs to different regions of the oocyte cytoplasm. This process is initiated through bi-directional signalling between the oocyte and the overlying follicle cells (Schüpbach, 1987). While the localised mRNAs and some of the signalling components have been studied in detail, many of the genes involved in these processes are still unknown (Nilson and Schüpbach, 1999; van Eeden and St Johnston, 1999).

*grk* mRNA is localised in early oocytes in a posterior crescent between the nucleus and the follicle cells (Neuman-Silberberg and Schüpbach, 1993), thus targeting the Grk transforming growth factor α (TGFα) signal only to the adjacent follicle cells. The Grk signal is probably the ligand for Torpedo/DER, an epidermal growth factor receptor (EGFR) (Gonzalez-Reyes et al., 1995; Neuman-Silberberg and Schüpbach, 1993). Grk instructs 200 terminal follicle cells to adopt posterior instead of default anterior fates (Gonzalez-Reyes and St Johnston, 1998). Posterior, anterior and main body follicle cells originate from the same group of cells that divides five or six times before stage 6 and has equivalent columnar epithelial morphology up to stage 9 (Gonzalez-Reyes and St Johnston, 1998). However, anterior and posterior follicle cells express distinct cell fate markers (Deng and Bownes, 1998; Fasano and Kerridge, 1988; Micklem et al., 1997).

Once the Grk signal is received, an unknown signal is sent from the posterior follicle cells back to the oocyte, repolarising the oocyte microtubules (MTs). MT organisation and polarity have been visualised in fixed material with anti-Tubulin antibodies (Theurkauf et al., 1992) and β-galactosidase (βgal) fusions to MT-dependent motor domains (Clark et al., 1994; Clark et al., 1997) as well as a TauGFP fusion in living oocytes (Micklem et al., 1997). Before stage 7, a microtubule organising centre (MTOC) is located at the posterior of the oocyte, where the minus ends of MTs are localised. At stage 7, the posterior MTOC disassembles, a diffuse anterior MTOC forms and plus ends of MTs are found at the posterior. The polarity of MTs determines the site of localisation of different mRNAs in the oocyte. *bicoid (bcd)* mRNA is localised to the anterior of the oocyte, leading to a morphogenetic gradient of Bcd protein in the embryo (Driever and Nüsslein-Volhard, 1988). *osk* mRNA is localised at the posterior of the oocyte and embryo and specifies the future germ cells (Ephrussi et al., 1991).

The Grk signal also initiates formation of the dorsoventral (DV) axis when the oocyte nucleus moves from the posterior to the dorsoanterior corner. *grk* transcripts then become tightly
localised near the nucleus, so that Grk signalling instructs only the overlying follicle cells to adopt dorsal fates (Nilson and Schüpbach, 1999; van Eeden and St Johnston, 1999). The specification of appropriate populations of follicle cells along the DV axis leads to the secretion of egg shell structures such as the dorsal appendages. Later in development the embryonic DV axis is formed by signalling from ventral follicle cells, which leads to the formation of a graded nuclear-cytoplasmic distribution of Dorsal protein (Anderson, 1998).

Although the posterior polarising signal remains unidentified, a number of known genes are required for the process. Protein kinase A (PKA) is likely to be part of the machinery that receives the signal in the oocyte (Lane and Kalderon, 1994; Perrimon, 1994) but is not specific to this process. Mago nashi (Mago) is required for oocyte repolarisation and has an independent function in osk mRNA localisation. However, Mago is a novel nuclear protein that is ubiquitous in the egg chamber, and its role in signalling is unknown (Micklem et al., 1997; Newmark et al., 1997). Notch-Delta signalling is required among the posterior follicle cells to limit the number of polar posterior follicle cells that express Fasciclin III; it is also required earlier in oogenesis (Larkin et al., 1996). Notch is required for the specification of posterior follicle cell identity (Gonzalez-Reyes and St Johnston, 1998), and is therefore required indirectly for the generation of the polarising signal to the oocyte (Ruohola et al., 1991), rather than being directly involved in the signal itself. Two other neurogenic proteins, Brainiac (Brn) and Egghead (Egh) are required in the oocyte for follicle cell integrity, and it has been suggested that both proteins may interact with Notch and EGF-signalling (Goode et al., 1996a; Goode et al., 1996b). However, it is not known whether they are required for the polarising signal. Lammin A is a component of the extracellular matrix that is expressed and required in the posterior follicle cells for the polarising signal (Deng and Ruohola-Baker, 2000). A better understanding of the events associated with the polarising signal awaits the identification of the signal itself.

Here, we identify a new allele of Mer by screening a collection of temperature sensitive (ts) lethal alleles for defects in grk mRNA localisation and we show that Merlin functions in axis specification during oogenesis. Drosophila Mer was previously cloned by degenerate PCR (McCartney and Fehon, 1996) and mutations isolated by reverse genetic methods (Fehon et al., 1997). The human homologue is a tumour suppressor called neurofibromatosis-2 (NF2), which encodes Merlin (Moesin Ezrin Radixin Related Protein) (McCartney and Fehon, 1996). Merlin and Ezrin-Radixin-Moesin (ERM) proteins are members of the 4.1 family of proteins thought to link actin to transmembrane proteins (Mangle et al., 1999; Tsukita et al., 1994) and Drosophila Merlin is apically localised in follicle cells (McCartney and Fehon, 1996). We show that Merlin is required only within the posterior follicle cells for mRNA localisation and axis specification in the oocyte. Merlin functions downstream of the Grk signal from the oocyte, but is only required if the posterior follicle cells receive the Grk signal. Merlin has no role in Notch-Delta signalling between the follicle cells, but is required upstream of the unknown polarising signal back to the oocyte. Merlin is also required non-autonomously in posterior follicle cells to limit their proliferation and maintain their polarity. We propose that Merlin functions by apically targeting the unknown polarising signal that initiates axis specification.
Sigma) or anti βh-spectrin (1:200) in PBTX with 2% BSA overnight at 4°C, followed by AlexaFluor594- and AlexaFluor488-coupled secondary antibodies, respectively (Molecular Probes).

RESULTS

Identification of a temperature-sensitive mutation that disrupts mRNA localisation and oocyte nuclear migration

To identify new genes required for axis specification, we screened a collection of X-linked ts lethal mutations generated by selecting for male lethality at 29°C and viability at 21°C. We collected homozygous female progeny at 21°C from 73 viable ts lethal lines, shifted to 29°C for 3 days and performed grk RNA in situ hybridisation on ovaries. In wild type or yw67g controls at 29°C or in all strains at 21°C, the oocyte nucleus migrates correctly to the antero-dorsal corner of the oocyte with grk mRNA localising between the nucleus and the overlying future dorsal follicle cells (Fig. 1A). In one line, yw67g |l(1)ts594 |l(1)ts594|, 55% (n=89) of oocyte nuclei fail to migrate and grk mRNA localises at the posterior after stage 8 (Fig. 1B). The remaining 45% of cases were similar to wild type, l(1)ts594 at 21°C (Fig. 1A) and the same genetic background yw67g (yw) chromosome at 29°C. In all subsequent experiments, similar controls were carried out, showing that the phenotype was not due to the temperature shift itself or the genetic background.

We also performed in situ hybridisation on l(1)ts594 ovaries to detect bicoid (bcd) and oskar (osk) mRNA. We found that in 83% (n=47) of stage 9 or 10A mutants at 29°C, bcd mRNA is localised at the posterior as well as its normal accumulation in an anterior ring (Fig. 1C,D). In 89% (n=32) of stage 9 and 10A mutants, osk mRNA is mislocalised at the centre of the oocyte (Fig. 1F) instead of its normal posterior localisation (Fig. 1E).

To test whether the defects in the oocyte are primarily due to a defect in MT organisation, we examined MT polarity. We used Kin:βgal, a plus end-directed MT motor fusion that leads to βgal accumulation at the posterior of the oocyte (Clark et al., 1994). We also used Nod:βgal, a MT motor fusion that leads to βgal accumulation at the anterior, where the minus ends of MTs are thought to localise (Clark et al., 1997). The βgal motor fusions indicate that prior to stage 7, there is an MTOC at the posterior (data not shown). In wild-type oocytes after stage 7, the posterior MTOC disassembles, a diffuse MTOC appears at the anterior (data not shown). In wild-type oocytes after stage 7, the posterior MTOC fails to disassemble at the posterior and a second diffuse MTOC forms at the anterior (Fig. 2B). This leads to a symmetric organization of MTs, with their plus ends at the centre of the oocyte (Fig. 2D) and minus ends at the anterior and posterior (Fig. 2B). We also examined the overall distribution of MTs using a maternally expressed TauGFP line showing the highest concentration of MTs at the anterior cortex of wild-type oocytes (Micklem et al., 1997). We observed a similar Tau-GFP distribution in l(1)ts594 oocytes at 21°C (Fig. 2E). In l(1)ts594 at 29°C Tau-GFP showed an abnormally high level at the posterior, consistent with a failure to disassemble the posterior MTOC (Fig. 2F). We conclude that the mislocalisation of mRNA and failure of the oocyte nucleus to relocate in l(1)ts594 oocytes are due primarily to defects in MT organisation.

l(1)ts594 is a strong loss-of-function allele of Mer

In order to determine the gene mutated in l(1)ts594, we mapped the mutation. Complementation analysis against deficiencies showed that the mutation lies in one of two gaps in the available deficiencies on the X chromosome, 18A2-A5 or 18D1-18E1-2 and recombination mapping showed that the lethality and oocyte phenotype both map to 18D-18E. Complementation analysis with all the available alleles in the region showed that three lethal alleles of Merlin (Mer1, Mer2 and Mer4) (LaJeunesse et al., 1998) failed to complement the lethality of l(1)ts594 at 29°C. A fusion of the Mer full-length cDNA with GFP and a cosmid containing a genomic DNA fragment including Mer are both able to fully complement the lethality, oocyte nuclear migration defects and mRNA mislocalisation of l(1)ts594 (data not shown), suggesting that l(1)ts594 is a ts allele of Mer.

Mer is the closest Drosophila homologue of human Merlin, a member of the ERMs family encoded by the NF2 tumour suppressor (Mangeat et al., 1999). ERM proteins are thought to link actin with transmembrane proteins at the cell membrane (Turunen et al., 1998) and may play a role in signalling (Mangeat et al., 1999). We sequenced the entire coding regions
Merlin protein has previously been shown to be expressed in Notch signalling (Fehon et al., 1996), but its function was only studied later in development (LaJeunesse et al., 1998; McCartney et al., 2000). To determine where Merlin functions in egg chambers, we studied the expression of different follicle cell populations and the oocyte, we studied the oogenesis phenotype of various other allelic combinations of Mer. We found similar defects in all the allelic combinations studied. Flies homozygous for Mer^{ts1}, and flies with Mer^{ts1} over a null allele (LaJeunesse et al., 1998) (Mer^{ts1}/Mer^{b}) showed almost identical phenotypes. Mer^{ts1} over a weak allele (Fehon et al., 1997) (Mer^{ts1}/Mer^{b}) showed a slightly reduced frequency of the oogenesis phenotype (data not shown). From these results, and the fact that a Mer transgene fully complements the Mer^{ts1} phenotype (data not shown), we conclude that Mer^{ts1} is a very strong loss-of-function allele, similar to a null.

**Merlin is not required in the germline, or for Grk or Notch signalling**

Merlin protein has previously been shown to be expressed in the oocyte and in posterior follicle cells (McCarty and Fehon, 1996), but its function was only studied later in development (LaJeunesse et al., 1998; McCartney et al., 2000).

To determine whether the phenotype we observed in Mer^{ts1} mutants was typical of existing loss-of-function Mer alleles, we studied the oogenesis phenotype of various other allelic combinations of Mer. We found similar defects in all the allelic combinations studied. Flies homozygous for Mer^{ts1}, and flies with Mer^{ts1} over a null allele (LaJeunesse et al., 1998) (Mer^{ts1}/Mer^{b}) showed almost identical phenotypes. Mer^{ts1} over a weak allele (Fehon et al., 1997) (Mer^{ts1}/Mer^{b}) showed a slightly reduced frequency of the oogenesis phenotype (data not shown). From these results, and the fact that a Mer transgene fully complements the Mer^{ts1} phenotype (data not shown), we conclude that Mer^{ts1} is a very strong loss-of-function allele, similar to a null.

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To test whether Merlin is required within the somatically derived posterior follicle cells to receive the Grk signal from the oocyte, we studied the expression of different follicle cell markers in Mer^{ts1} egg chambers. The results show that Mer^{ts1} posterior follicle cells receive the Grk signal correctly, as they express posterior and not anterior markers (Fig. 3A-D). We conclude that Merlin is not required for any aspect of Grk signalling or its reception in the posterior follicle cells. Merlin is also not required for Notch signalling among the posterior follicle cells, which is required to specify the correct number of posterior cells (Gonzalez-Reyes and St Johnston, 1998).

We also tested whether Merlin is required for the formation or identity of other types of follicle cells by analysing markers for different follicle cell populations in Mer^{ts1}. These included a marker for border cells, stalk cells and polar follicle cells. Our results show that Merlin is not required for the correct...
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specification or development of any subgroup of follicle cells, and is not required for Notch signalling among the follicle cells, which limits the number of polar follicle cells to two (Fig. 3E,F). Merlin is therefore likely to be required for cell communication between the follicle cells and oocyte, downstream of Grk and upstream of the unknown polarising signal from the posterior follicle cells to the oocyte.

Merlin acts as a tumour suppressor in posterior follicle cells and is required for their polarity

We observed that the posterior follicle cells in fixed (Figs 3D-F, 4D-F,H) and living (data not shown) Mer ts1 egg chambers often have a slightly disrupted morphology. To study these defects in more detail, we covisualised actin and DNA to highlight each cell and its boundaries (Fig. 4A,D). Posterior follicle cells in controls have a uniform columnar appearance characteristic of epithelial sheets (Fig. 4A). However, after stage 6, Mer ts1 egg chambers have a double layer of follicle cells only at the posterior where follicle cells are in contact with the oocyte (Fig. 4D). To determine whether the double layer of posterior follicle cells is due to overproliferation, we counted the number of cells using three-dimensional microscopy and found a twofold increase in the number of posterior follicle cells, but no changes in other follicle cells (data not shown).

To determine whether the overproliferation of posterior follicle cells is accompanied by polarity defects, we studied MT polarity by covisualising DNA, the nuclear envelope and centrosomes (Fig. 4B,C,E,F). In control egg chambers, most
from non-mosaic homozygous 
grk2E12 (mutants. We found that even a hypomorphic allele of 
chambers showed a strong 
Grk signal, we examined the follicle cells of 
Mer egg chambers are dependent on receiving the 
polarity in 
receive signals to the oocyte. 
mutants, the apical surface of posterior follicle cells contacts 
follicle cells (Fig. 4H). These results suggest that in Mer 
mutants, the apical surface of posterior follicle cells contacts 
the oocyte correctly, and is probably competent to send and 
receive signals to the oocyte.

To determine whether the defects in cell proliferation and 
polarity in Mer egg chambers are dependent on receiving the 
Grk signal, we examined the follicle cells of Mer, grk double 
mutants. We found that even a hypomorphic allele of grk 
(grk2E12) suppresses the Mer posterior follicle cell phenotype 
etirely (data not shown). We conclude that Merlin is required 
only in cells that receive the Grk signal and is not a constitutive 
factor required for cell polarity and proliferation.

Merlin is required non-autonomously in posterior 
follicle cells

To test directly whether Merlin is required only in posterior 
follicle cells, we used genetic mosaic analysis with the 
FRT/FLP system to make clones of homozygous Mer follicle 
cells located at posterior, anterior or central positions (Fig. 5A- 
I). We found that Merlin is required only in the posterior 
follicle cells for their correct morphology and migration of the 
oocyte nucleus. We produced Mer clones using MerFRT/nlsGFP,FRT; en-Gal4,UASFLP females. Mer is a 
hozygous viable but sterile allele (Fehon et al., 1997) and 
en-Gal4,UASFLP expresses FLP recombinase at very high 
levels only in the follicle cells (Duffy et al., 1998). We examined a total of 43 egg chambers with Mer clones, of 
which 29 were particularly revealing and analysed in detail. Of 
these, one egg chamber had follicle cells that were entirely 
Mer (Fig. 5A-C), and three egg chambers had large mutant 
clones covering all the posterior (data not shown). These egg 
chambers showed a strong Mer phenotype indistinguishable 
from non-mosaic homozygous Mer mutants. 21 egg chambers 
without a 
Mer phenotype (data not shown). We conclude that Merlin is required only in posterior follicle cells.

To test whether Merlin is required cell autonomously to limit 
the proliferation and polarity of posterior follicle cells, we 
studied four egg chambers in which the follicle cells were 
Mer, except for one or more very small Mer+ clones in the 
posterior follicle cells. Such egg chambers showed complete 
rescue of the Mer phenotype when sufficient Mer+ cells were 
present (Fig. 5D-F), indicating that Merlin acts cell non-
autonomously among the posterior follicle cells. One of these 
clones had a single Mer+ cell at the posterior tip surrounded 
by Mer cells, showing that a single Mer+ cell is able to 
rescue the overproliferation phenotype up to a distance of about 
six cell diameters (Fig. 5G-I). While the single Mer+ cell was 
not able to rescue the oocyte nuclear migration defect, several 
small Mer+ clones were sufficient to do so. We conclude that 
Merlin is required non-autonomously in the posterior follicle 
cells to limit their proliferation.

Merlin is not required during embryogenesis

To test whether Merlin is required for embryogenesis we 
analysed the hatch rate of eggs laid by Mer mothers. At 
29°C, 74% of eggs (n=100) hatch and develop normally until 
third instar larvae, compared with a hatch rate of 94% for 
Mer at 21°C and yw 67g at 29°C (a difference of 21%). All 
the embryos have abdominal cuticle defects similar to 
Mer eggs hatch, since 
Initially, it was surprising that most Mer eggs hatch, since 
mislocalised bed mRNA would be expected to disrupt AP axis 
specification and cause embryonic lethality. However, we 
found that bed mRNA, which was mislocalised at the posterior, 
partially or completely relocalised in older egg chambers (Fig. 
7A-D). Consequently, Mer embryos have completely normal 
bed localisation (Fig. 7E,F). The near normal hatch rate of Mer 
embryos was also initially surprising because 55% (n=89) of Mer 
mutants have misplaced oocyte nuclei and mislocalised grk 
mRNA, which would lead to embryonic lethality. However, we
found that only 11% (n=158) of eggs laid by Mer<sup>ts1</sup> mothers have strong dorsoventral defects (Fig. 7H,K,L), and the other defective egg chambers degenerate in females after stage 10A (data not shown). It is likely that egg chambers with mislocalised osk mRNA also degenerate in the mothers, explaining why there is a lower percentage of osk mRNA localisation defects in embryos compared with oocytes.

We conclude that Mer eggs hatch at a slightly lower frequency than controls because of abdominal and dorsoventral defects that originate during oogenesis, rather than a direct requirement for Merlin in embryos. Therefore, Merlin is not required for embryogenesis and much of larval development.

DISCUSSION

We have shown that Merlin is required for the signal that initiates axis specification. Merlin is also required non-autonomously for signalling among the posterior follicle cells that limits their proliferation and maintains their polarity. Merlin is not required for other signals within the posterior follicle cells or in other parts of egg chambers and embryos.

Taking our data in the context of previous work, we propose that Merlin is involved in apical targeting of the unknown signal that initiates axis specification in the oocyte. Merlin is a member of the ERM/4.1 family of proteins and, in Drosophila, it is localised to the apical membrane of follicle cells and in the germline (McCartney and Fehon, 1996). ERM family members are thought to function as linkers between the cytoskeleton and the apical membrane, and they are probably required for apical targeting of signals, maintenance of epithelial adhesion, apical-basal polarity and to limit cell proliferation (Vaheri et al., 1997).

The overproliferation of the posterior follicle cells is consistent with overproliferation of mutant Mer cells seen in imaginal discs and with the function of human Merlin as a tumour suppressor causing neurofibromatosis-2. The changes in cell polarity we observe are also common in many other types of tumours. Interestingly, as in other Drosophila tissues (LaJeunesse et al., 1998), the Mer phenotypes we have studied are more similar in character to benign tumours seen in individuals with neurofibromatosis-2 than to the aggressive tumours produced in the mouse model (McClatchey et al., 1998). However, it is not known whether Merlin is required during mammalian oogenesis. We speculate that human Merlin may function in a similar non-autonomous manner in response to particular signals such as TGF-α, which is known to be expressed in mammalian oocytes (Vaughan et al., 1992). Indeed, many parallels may exist between mammalian and fly oogenesis in respect of communication between the oocyte and follicle cells (Deng et al., 1997).

Is Merlin directly involved in signals that initiate axis specification?

We have shown that Merlin has a more specific and restricted function than previously thought, as it is required only in cells that receive the posterior Grk signal, despite being expressed more widely in egg chambers (McCartney and Fehon, 1996). Interestingly, the dorso-anterior follicle cells do not require Merlin, despite receiving the Grk signal.

Our data show that Merlin is required downstream of Grk but upstream of the unknown polarising signal. We propose that the effect of Merlin on the polarising signal is not indirectly due to the overproliferation and subtle changes in the polarity of the posterior follicle cells. β<sub>III</sub>-Spectrin is correctly distributed in Mer follicle cells adjacent to the oocyte, despite the centrosomes being disorganised and the second layer of follicle cells showing mislocalised β<sub>III</sub>-spectrin. Therefore, the inner layer of posterior follicle cells are probably competent to...
send the polarising signal in Mer mutants. Furthermore, some Mer egg chambers were found in which the polarising signal was not received, despite the posterior follicle cells showing no apparent defects in their proliferation or polarity (data not shown). We also found that brn mutant egg chambers show a similar specific morphological disruption of posterior follicle cells to that seen in Mer mutants (Goode et al., 1996a; Goode et al., 1996b) but brn mutations do not lead to any defects in oocyte axis specification (data not shown). Therefore, the morphological disruption of the posterior follicle cells in itself is not likely to be responsible for perturbing the unknown signal to the oocyte. Instead, we propose that Merlin may have a more direct role in targeting the polarising signal to the apical surface of posterior follicle cells.

What signals are disrupted by Mer mutations?

Since many genes involved in signalling among the follicle cells and between the oocyte and follicle cells are unknown, it is difficult to be certain which signals might be disrupted by Mer mutations. Nevertheless, our data conclusively rule out a role for Merlin in a number of known signalling processes. Merlin is not required for receiving the Grk signal via Torpedo, an EGF-like receptor, by the posterior or dorso-anterior follicle cells. Merlin is also not required for lateral inhibition via Notch-Delta signalling among the posterior follicle cells, that determines the correct number of posterior, polar posterior follicle cells and stalk cells between egg chambers. Nor is Merlin required for many kinds of essential signalling pathways throughout embryogenesis. Merlin is unlikely to play a direct role in the presumptive Egh/Brn signal from the oocyte, as these proteins are required in the oocyte and not in the follicle cells. Nevertheless, it is intriguing that the posterior follicle cells of N, brn or egh mutant egg chambers all show a similar overproliferation phenotype to Mer egg chambers. While it is possible that N, Egh or Brn are in some way related in function to Merlin, further experiments will have to be performed to explore these issues.

Our results show that Merlin is required for two distinct processes involving signalling, but we cannot distinguish whether the two processes depend on a single signal or two distinct signals. For example, the restriction of posterior follicle cell proliferation could require the same unknown signal that initiates MT repolarisation in the oocyte. Both processes could depend on the same signal secreted into the space between the follicle cells and oocyte. Indeed, it is intriguing that Merlin egg chambers have MT polarity defects in both the oocyte and the posterior follicle cells. However, further progress awaits the identification of the signal or signals involved.

The identity of the polarising signal is unknown, but some genes are known to be required for the signal, including PKA (Lane and Kalderon, 1995), Mago (Micklem et al., 1997) and Laminin A. Merlin is unlikely to be required for PKA and Mago functions as they are required in the oocyte. In contrast, Laminin A is expressed and required in posterior follicle cells as a component of the extracellular matrix (Deng and Ruohola-Baker, 2000). It is tempting to speculate that Merlin and Laminin A could be functionally linked as specialised structural components required specifically in the posterior follicle cells for the transduction of the polarising signal.

It is interesting to ask how many additional components are required for axis specification in the oocyte but it is not possible to estimate this number from our results. However, the fact that we identified even one mutation required for this process out from only 73 ts alleles, strongly argues that there are many more unrecognised genes required for axis formation.

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