Role of Bicaudal-D in patterning the Drosophila egg chamber in mid-oogenesis

Andrew Swan
University of Windsor

B. Suter

Follow this and additional works at: https://scholar.uwindsor.ca/biologypub

Recommended Citation
https://scholar.uwindsor.ca/biologypub/1120

This Article is brought to you for free and open access by the Department of Biological Sciences at Scholarship at UWindsor. It has been accepted for inclusion in Biological Sciences Publications by an authorized administrator of Scholarship at UWindsor. For more information, please contact scholarship@uwindsor.ca.
Role of Bicaudal-D in patterning the Drosophila egg chamber in mid-oogenesis

Andrew Swan and Beat Suter
Department of Biology, McGill University, 1205 Dr Penfield Avenue, Montreal, PQ, Canada, H3A 1B1

SUMMARY

The Bicaudal-D (Bic-D) gene is required early in Drosophila oogenesis for the differentiation of an oocyte from one of a cluster of 16 interconnected germarial cells. To analyze the role of Bic-D later in oogenesis, we have constructed Drosophila lines in which Bic-D expression is under the control of the hsp70 promoter. In these flies, Bic-D activity can be induced early in oogenesis, allowing an oocyte to be made. Then, by shifting females to non-inducing conditions, Bic-D levels are depleted for the remainder of oogenesis. Using this system, we find that Bic-D is indeed required in the later stages of oogenesis. In ovaries from mutant females, oocyte growth is reduced, apparently due to defects in nurse-cell-to-oocyte transport. Smaller oocyte size results in the misalignment of follicle cells and the underlying germ line, leading to ventralization of dorsal follicle cells and to defects in centripetal cell migration. In addition, we show that Bic-D is required for the localization of specific mRNAs at both the anterior and posterior of the oocyte.

Key words: Drosophila, oogenesis, cytoskeleton, axis formation, RNA localization, Bicaudal-D (Bic-D), egg chamber, follicle cell, nurse cell

INTRODUCTION

Asymmetric localization of patterning factors within cells often underlies developmental patterning, and Drosophila oogenesis has proven to be a valuable system for studying how such asymmetries arise. The Drosophila ovary has been divided into 3 germarial regions and 13 vitellarial stages. In the gerarium, a cytoblast undergoes four incomplete divisions to produce a 16-cell cyst in which all cells are connected by cytoplasmic bridges. Beginning in region 2b, a microtubule-organizing center (MTOC) appears in one of these cells and extends microtubules through the ring canals into the other 15 cells. Specific mRNAs and proteins accumulate in this cell and it differentiates as an oocyte. The other 15 cells become nurse cells and function in providing material for the growing oocyte (reviewed in Cooley and Theurkauf, 1994). Microtubule-destabilizing drugs disrupt the accumulation of factors in the pro-oocyte and result in the failure to differentiate an oocyte (Koch and Spitzer, 1983; Theurkauf et al., 1993). This evidence supports a model in which a polarized microtubule network directs the transport of specific factors into the future oocyte. Among these would be factors that promote oocyte differentiation.

During stages 7 and 8, the oocyte MTOC disappears and microtubules reorganize within the oocyte such that minus ends are found mainly at the anterior and plus ends extend towards the posterior (Theurkauf et al., 1992). This polarized microtubule network is thought to direct the anterior and posterior transport of specific factors within the oocyte (see Cooley and Theurkauf, 1994). Transcripts encoding the anterior morphogen, bicoid, accumulate at the anterior of the oocyte in stage 8 and remain at this site throughout oogenesis. Several other transcripts, including Bic-D, orb and fs(1)K10 mRNAs also accumulate at the anterior of the oocyte at this time and remain at this location until late in stage 10. Also in stage 8, staufen protein and oskar (osk) mRNA migrate together to the posterior pole of the oocyte where osk is then translated and functions to recruit other posterior factors. Accumulation of these anteriorly and posteriorly localized transcripts is disrupted by treating egg chambers with microtubule-destabilizing drugs (Pokrywka and Stephenson, 1991, 1995; Clark et al., 1994), suggesting that microtubule-based transport and/or anchoring is involved in their localization. This model is supported by the observation that in females expressing a transgenic kinesin-β-gal fusion protein, the fusion protein accumulates transiently at the posterior pole of the oocyte at the same time that osk mRNA and staufen protein are first detected there (Clark et al., 1994).

During the stages when A/P patterning is set up, the first signs of D/V polarity in the egg chamber are detected. In stage 8, grk mRNA associates with the oocyte nucleus and moves with it to an anterior/cortical position within the oocyte. Localized grk protein is thought to signal overlying follicle cells through the top receptor, initiating a signal transduction cascade involving members of the Ras/Raf/MAP Kinase pathway (reviewed in Schüpbach and Roth, 1994). Late in oogenesis, the follicle cells that surround the maturing egg produce the vitelline membrane and chorion. Dependent on grk/top signaling, the dorsal anterior follicle cells produce specialized dorsal chorion structures, the dorsal appendages and the operculum.

The product of the Bicaudal-D (Bic-D) gene may play a
role in a number of patterning processes during oogenesis. In ovaries from females homozygous or hemizygous for loss-of-function alleles of Bic-D, no oocyte is made and all 16 cells of the germarial cyst adopt a nurse cell fate (Mohler and Wieschaus, 1986). The MTOC, which normally forms in the oocyte, is not detected in these mutants and oocyte-specific mRNAs fail to accumulate in a single cell (Suter and Steward, 1991; Ran et al., 1994; Theurkauf et al., 1993). These observations may point to a role for Bic-D in microtubule-based transport or anchoring early in oogenesis.

The phenotypes produced by two dominant alleles of Bic-D suggest that this gene also functions later in oogenesis, in both A/P and D/V patterning of the egg chamber. Females carrying either Bic-D\textsuperscript{71.34} or Bic-D\textsuperscript{H1E49} produce embryos missing anterior structures and instead possessing a mirror-image duplication of posterior structures (Mohler and Wieschaus, 1986). This defect appears to result from a mislocalization of osk mRNA to the anterior pole of the oocyte late in oogenesis (Ephrussi et al., 1991; Kim-Ha et al., 1991). This result, along with the finding that the earlier localization of osk mRNA to the pro-oocyte is blocked in Bic-D\textsuperscript{null} mutants (Ran et al., 1994), may indicate a role for Bic-D in osk mRNA localization later in oogenesis. The two dominant Bic-D alleles also display a recessive fused dorsal appendage phenotype similar to that produced by weak alleles of grk and top (Mohler and Wieschaus, 1986). Therefore, there is evidence that Bic-D is involved in the establishment of both primary axes.

Bic-D\textsuperscript{71.34} and Bic-D\textsuperscript{H1E49} are dominant gain-of-function mutations and thus their phenotypes may not be indicative of actual Bic-D function. To understand the role of Bic-D in patterning of the egg chamber, it is essential to determine the loss-of-function phenotypes for Bic-D during these stages. So far this has not been possible because all recessive alleles of Bic-D prevent the formation of an oocyte. Study of the later requirements for Bic-D would be possible if Bic-D activity could be provided early in oogenesis, permitting an oocyte to be made, but then removed for the remainder of oogenesis. We have accomplished this by introducing an inducible Bic-D transgene into female Drosophila otherwise lacking Bic-D activity. Using this method, we find that Bic-D is required for oocyte growth and both D/V and A/P patterning of the egg chamber. Our results also provide evidence for a prepatterning of follicle cell fates independent of the underlying oocyte.

MATERIALS AND METHODS

Construction of Bic-D mid-oogenesis mutants (Bic-D\textsuperscript{mom})

The 3.6 kb Clal/EcoRI fragment from Bic-D cDNA c18 (Suter et al., 1989), which includes the entire open reading frame, was cloned as a leader fusion behind the I\textsuperscript{a} promoter in the pElba vector (a gift from Paul Schedl, Princeton). This vector possesses scs and scs' boundary domains to minimize chromosomal position effects (Kellum and Schedl, 1991). The fusion gene was then inserted into a pCaSpeR vector and introduced into flies by P-element-mediated transformation. The results described here are from experiments performed on one line, P[w\textsuperscript{hs}Bic-D\textsuperscript{-}]94/4, though the same phenotypes were seen with two other independently derived lines. To generate Bic-D mid-oogenesis mutant (Bic-D\textsuperscript{mom}) females, progeny from the cross, w;Df[2L]/TW119/CyO; P[w\textsuperscript{hs}Bic-D\textsuperscript{-}]94/+ x w;Bic-D\textsuperscript{+/CyO} were given two 37°C heat shocks of 30 minutes per day starting late in the 3rd larval instar or at the early pupal stage, and lasting for 3 to 4 days. The lethality associated with Bic-D\textsuperscript{null} mutations (Ran et al., 1994) is rescued by this induction of the transgene. These flies were then transferred to 18°C and Bic-D\textsuperscript{+/Df[2L]/TW119}; P[w\textsuperscript{hs}Bic-D\textsuperscript{-}]94/+ (Bic-D\textsuperscript{mom}) females were selected and examined for oogenesis and egglay phenotypes at various times after the shift. Sibling females of the genotype, w;Bic-D\textsuperscript{+} (or Df[2L]/TW119/CyO; P[w\textsuperscript{hs}Bic-D\textsuperscript{-}]94/+ were processed in parallel and used as controls in all experiments except in the protein blot analysis and immunostaining with anti-Bic-D antibodies. For these experiments, Oregon\textsuperscript{R} flies were used as controls. For all experiments involving examination of ovary phenotypes, females were aged 2 to 4 days on standard food with live yeast prior to ovary dissection.

Protein blot analysis

Protein blots were performed as described in Suter and Steward (1991) using ovary extracts pooled from five females for each sample. All Bic-D protein detected in these ovaries is produced by the transgene, since the Bic-D\textsuperscript{+} allele makes very low levels of a truncated protein (Ran et al., 1994).

Immunostainings

Ovaries from Bic-D\textsuperscript{mom} and Oregon\textsuperscript{R} females, collected at various times after shift to 18°C, were fixed and immunostained as previously described (Suter and Steward, 1991). To control for variability in fixation or staining, mutant and control ovaries were processed together in the same tube. Either control or mutant ovary pairs were separated into two to allow them to be distinguished later. Pictures were taken from single optical sections using a Leica confocal laser scanning microscope.

Ovary RNA in situ hybridizations

RNA in situ hybridizations were performed as described previously (Suter and Steward, 1991). Control and mutant ovaries were processed together.

Determination of chorion phenotypes

Eggs were collected from overnight egg lays and classified according to the severity of their chorion phenotype as described in Fig. 2. For photographing eggs with representative chorion phenotypes, eggs were mounted in Hoyers medium and photographed under dark field as described in Wieschaus and Nüsslein-Volhard (1986).

Enhancer trap detection

The BB127 and BB142 enhancer trap lines are described in Schüpbach and Roth (1994). The BB142 enhancer trap insertion on 2L was recombined onto a Bic-D\textsuperscript{+} chromosome and crossed to Df[2L]/TW119/CyO; P[w\textsuperscript{hs}Bic-D\textsuperscript{-}]94/+. The 3rd chromosome BB127 insert was crossed into a Bic-D\textsuperscript{mom} background. β-gal detection was performed as in Lis et al. (1983).

Egg chamber size analysis

Control and Bic-D\textsuperscript{mom} egg chambers were fixed in 4% paraformaldehyde in PBS + 0.2% Tween and stained with rhodamine phalloidin (Molecular Probes). Phalloidin labels actin at cell membranes and, therefore, allows us to easily trace cells. Individual egg chambers were scanned using a Leica confocal laser scanning microscope. Single images from optical sections giving the largest oocyte areas were measured using NIH imaging software. For each egg chamber, the ratio of oocyte area to total area (oocyte + nurse cells) was determined and, for all of the eggs of a given stage, these values were averaged. Egg chambers were staged according to follicle cell morphology.
RESULTS

An inducible Bic-D transgene allows examination of Bic-D requirements in late oogenesis

To examine the Bic-D loss-of-function phenotype in later oogenesis, we have placed the Bic-D coding sequence under the control of the hsp70 promoter and introduced this transgene into females otherwise lacking Bic-D activity. *scs* and *scs*′ boundary domains (Kellum and Schedl, 1991) flanking the transgene help ensure strict heat-shock control. When Bic-D is induced in these *Bic-Dnull*;*hs-Bic-D* females by providing them with regular heat shocks, they are fertile and lay mainly wild-type eggs. Total Bic-D protein levels in ovaries under these conditions are approximately half of wild-type levels (Fig. 1A). Immunostaining of these *Bic-Dnull*;*hs-Bic-D* ovaries with anti-Bic-D antibodies reveals that early in oogenesis, Bic-D protein is localized to the oocyte at levels similar to those seen in wild type (Fig. 1B). Stage 8 and later egg chambers, however, contain very low levels of Bic-D protein in the germ line (data not shown), possibly indicating that the hsp70 promoter is not as strongly inducible in the later stages of oogenesis. In addition to expression of Bic-D in the germ line, induction of the transgene also directs the expression of Bic-D in the somatically derived follicle cells which surround the 16-cell cyst. This somatic expression of Bic-D appears to have no adverse effect on oogenesis since no mutant phenotypes are observed upon induction of the transgene in control females carrying one copy of the endogenous Bic-D gene (data not shown).

Within one and a half days after shifting flies to non-inducing conditions (18°C), Bic-D protein levels fall noticeably and, by 5.5 days, Bic-D is almost undetectable by protein blotting or immunostaining (Fig. 1A,B). Correlating with a reduction in Bic-D protein levels after shift to 18°C, egg chambers start to display a 16-nurse-cell phenotype like that observed in Bic-D recessive mutants. After 12 to 13 days under non-inducing conditions, ovaries from these *Bic-Dnull*;*hs-Bic-D* females mainly consist of 16-nurse-cell chambers. Sometimes a few mature eggs are also present, representing the last egg chambers in which Bic-D levels were sufficient to make an oocyte.

Taken together, these results show that the *hs-Bic-D* transgene provides an inducible source of Bic-D activity, which can rescue the block at oocyte determination in *Bic-Dnull* females. Several days after shift to 18°C, we can examine egg chambers in which Bic-D activity was initially sufficient to permit oocyte determination, but was reduced or absent for the remainder of oogenesis. This allows us to determine the requirements for Bic-D after its role in oocyte determination. In the remainder of this paper, we will refer to *Bic-Dnull*; *hs-Bic-D* females shifted down to 18°C as Bic-D mid-oogenesis mutants or Bic-D mom and the oogenesis phenotypes that they display as Bic-D mom phenotypes.

Chorion defects in Bic-D mom egg chambers

Bic-D mom females lay eggs with phenotypes ranging from partially fused or fused dorsal appendages to reduced or absent dorsal appendages (Fig. 2A-C). In many cases, the dorsal appendage remnants are located more posteriorly on the eggshell than in wild type. In addition to dorsal appendage defects, another dorsal chorion structure, the operculum, is

---

Fig. 1. Induction of Bic-D protein in Bic-D null:hs-Bic-D (Bic-D mom) ovaries. (A) Protein blot of ovary extracts from wild-type and Bic-D mom females at various times after last induction of Bic-D. With the exception of lane 1, which contains 1/10 the amount loaded in lane 2, equal amounts of protein were loaded in each lane as determined by Ponceau S staining (not shown). 12 hours after the last heat shock, protein levels are approximately half of wild-type levels. By 5½ days, Bic-D protein is almost undetectable. (B) Confocal laser scanning images of wild-type and Bic-D mom egg chambers at different times after shift to 18°C. Under heat-shock conditions, the amount and distribution of Bic-D protein in early stage egg chambers is similar to that in wild type. Bic-D levels fall sharply following shift to 18°C, and only background signal can be detected by 5 days. Scale bar, 10 μm.
often reduced or missing, and the follicle cell imprints on the dorsal surface of the egg are rounded, resembling those normally seen on the ventral surface. Mutant females also lay eggs that are small and often lack anterior chorion (Fig. 2D).

Control results demonstrate that the chorion phenotypes that we observe in *Bic-D* null eggs are not due to trivial causes such as overexpression of *Bic-D*, mutations caused by insertion of the transgene or secondary effects resulting from heat shock. First, females heterozygous for *Bic-D*, with or without the transgene, and subjected to the same heat-shock regimen produce wild-type-looking eggs (data not shown). Second, all phenotypes are rescued by inducing *Bic-D* expression from the transgene (Fig. 2A,E). Third, the same phenotypes are observed in three independent transgenic lines (Fig. 2E)

Control results demonstrate that the chorion phenotypes that we observe in *Bic-D* null eggs are not due to trivial causes such as overexpression of *Bic-D*, mutations caused by insertion of the transgene or secondary effects resulting from heat shock. First, females heterozygous for *Bic-D*, with or without the transgene, and subjected to the same heat-shock regimen produce wild-type-looking eggs (data not shown). Second, all phenotypes are rescued by inducing *Bic-D* expression from the transgene (Fig. 2A,E). Third, the same phenotypes are observed in three independent transgenic lines (Fig. 2E and data not shown). Therefore, the dorsal appendage phenotypes are observed in three independent transgenic lines.

Second, all phenotypes are rescued by inducing *Bic-D* expression from the transgene or secondary effects resulting from heat shock. First, females heterozygous for *Bic-D*, with or without the transgene, and subjected to the same heat-shock regimen produce wild-type-looking eggs (data not shown). Second, all phenotypes are rescued by inducing *Bic-D* expression from the transgene (Fig. 2A,E). Third, the same phenotypes are observed in three independent transgenic lines (Fig. 2E and data not shown). Therefore, the dorsal appendage phenotypes are observed in three independent transgenic lines.

Control results demonstrate that the chorion phenotypes that we observe in *Bic-D* null eggs are not due to trivial causes such as overexpression of *Bic-D*, mutations caused by insertion of the transgene or secondary effects resulting from heat shock. First, females heterozygous for *Bic-D*, with or without the transgene, and subjected to the same heat-shock regimen produce wild-type-looking eggs (data not shown). Second, all phenotypes are rescued by inducing *Bic-D* expression from the transgene (Fig. 2A,E). Third, the same phenotypes are observed in three independent transgenic lines (Fig. 2E and data not shown). Therefore, the dorsal appendage phenotypes are observed in three independent transgenic lines.

The values on the graph represent the average severity of the chorion phenotype from a single egg collection. An average of 70 eggs were scored from each collection, 31 eggs for the smallest. Females carrying the *Bic-D* null allele, *hs-Bic-D-132* insert required heat shocks 4 times daily to rescue the fused dorsal appendage phenotype. Results with *Bic-D* null are similar to those obtained with *Bic-D* null (data not shown).

![Fig. 2. Ventralized chorion phenotypes produced by *Bic-D* null females. (A) Wild-type-looking eggshell produced by a *Bic-D* null 3 days after last induction of *Bic-D*. (B-D) Chorion phenotypes from *Bic-D* null eggs 10 days after last induction of *Bic-D*. (B) Fused dorsal appendages. (C) Reduced and posteriorly displaced dorsal appendages. (D) Open chorion. (E) Severity of chorion phenotypes as a function of time after the last induction of *Bic-D*. Eggs collected at various timepoints were classified and assigned a value according to the severity of their chorion phenotype: wild type=0, partially fused dorsal appendages=1, fused dorsal appendages=2, reduced or absent dorsal appendages=3, open chorion phenotype=4. The results for *Bic-D* null, *hs-Bic-D-94*, expressed as percentages (open circles on the graph) are as follows:

<table>
<thead>
<tr>
<th>Days after heat shock</th>
<th>% of eggs in each phenotypic class</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>39 59 2 0 0</td>
</tr>
<tr>
<td>1-2</td>
<td>36 59 4 2 0</td>
</tr>
<tr>
<td>2-3</td>
<td>38 58 3 1 0</td>
</tr>
<tr>
<td>3-4</td>
<td>24 52 13 8 2</td>
</tr>
<tr>
<td>5-6</td>
<td>3 27 24 27 19</td>
</tr>
<tr>
<td>9-10</td>
<td>3 19 20 49 24</td>
</tr>
</tbody>
</table>

The values on the graph represent the average severity of the chorion phenotype from a single egg collection. An average of 70 eggs were scored from each collection, 31 eggs for the smallest. Females carrying the *Bic-D* null allele, *hs-Bic-D-132* insert required heat shocks 4 times daily to rescue the fused dorsal appendage phenotype. Results with *Bic-D* null are similar to those obtained with *Bic-D* null (data not shown).
underlies the anteriormost columnar follicle cells, and it is these cells that normally appear to receive grk signal (Schüpbach and Roth, 1994). In stage 10A Bic-Dmom egg chambers, columnar follicle cells still extend over the posterior half of the egg chamber. However, the oocyte is smaller than in wild type and the margin between oocyte and nurse cells is displaced posteriorly relative to the border between flattened and columnar follicle cells (Fig. 3D). As a result, grk signal is displaced relative to the overlying follicle cells. This in turn results in a posterior displacement of follicle cell fates as seen in the expression of the dorsal/anterior follicle cell marker BB142 (Fig. 3F). This indirect effect on grk/top signaling could explain the failure to properly establish dorsal follicle cell fates and demonstrates that the establishment of D/V polarity in the egg chamber depends on the proper coordination between oocyte growth and follicle cell migration.

In stage 10 and later Bic-Dmom egg chambers, we often observe a mislocalization of the oocyte nucleus (Fig. 4A). Video analysis of mutant egg chambers reveals that the oocyte nucleus often moves within the oocyte, usually confined to the cortex, during the rapid ooplasmatic streaming that begins in stage 10B (data not shown). Some grk mRNA remains associated with the nucleus even when it is mispositioned (data not shown). This subsequent displacement of grk mRNA late in oogenesis may also affect follicle cell fate since BB142 expression, in addition to being displaced posteriorly, is frequently found at low levels in a broad range of follicle cells in stage 10B mutant egg chambers (Fig. 3F).

Dependent on grk/top signaling, dorsal follicle cells are thought to produce a signal required later for the restriction of ventral fates in the embryo, and therefore many mutants that disrupt the specification of dorsal follicle cell identity also disrupt the polarity of the embryo (reviewed in Schüpbach and Roth, 1994). However, no embryonic D/V patterning defects are observed in eggs from Bic-Dmom females. This could simply be due to the fact that most eggs laid by these mutants are not fertilized and those eggs with the most severe chorion defects are never fertilized (data not shown). Alternatively, it is possible that the follicle cells that receive dorsalizing signal in Bic-Dmom egg chambers, while unable to induce proper dorsal appendage formation, are still able to repress ventral fates in the embryo.

In addition to ventralized eggs, Bic-Dmom females produce eggs that are missing anterior chorion (Fig. 2D). Similar open chorion phenotypes have been described for a small number of maternal effect mutants (Schüpbach and Wieschaus, 1991). One of these, Bicaudal-C, also displays a dominant bicaudal phenotype and therefore these two genes share similar recessive and dominant phenotypes. As with the other known open chorion mutants, the Bic-Dmom phenotype appears to be a result of failed centripetal cell migration. Normally in stage 10B, the anterior columnar follicle cells overlying the oocyte/nurse cell boundary migrate between the oocyte and the nurse cells, separating the maturing egg from the nurse cells. The centripetal cells later contribute towards making the anterior portion of the egg shell. In Bic-Dmom egg chambers, centripetal cell migration is often incomplete, likely because nurse cells extend into the space where these cells normally would migrate, physically impeding their progress. Consistent with this interpretation, we often see egg chambers in which the nurse cells bordering the oocyte appear to have been displaced far enough posteriorly to allow centripetal cells to intercalate between them and the next set of nurse cells (Fig. 4B,C).

### Bic-D requirement in oocyte growth

The defects in D/V patterning and in centripetal cell migration in Bic-Dmom appear to be indirect consequences of a failure in oocyte growth. Throughout most of oogenesis the oocyte nucleus is largely transcriptionally inactive and oocyte growth occurs primarily by uptake of materials from other cells (King and Burnett, 1959). Oocyte growth early in oogenesis appears to occur via a microtubule-dependent process (Koch and Spitzer, 1983), while after stage 7 an actin-dependent and microtubule-independent transport of nurse cell contents into the oocyte has been observed (Bohrman and Biber, 1994). Also beginning in stage 8, the oocyte expands by uptake of yolk from the surrounding follicle cells and hemolymph.

To determine which aspect of oocyte growth is affected in Bic-Dmom egg chambers, we measured oocyte and egg chamber size in mutant and control egg chambers, and for each egg chamber we determined the ratio of oocyte to total egg chamber size (Table 1). This analysis reveals that in Bic-Dmom ovaries, oocyte size is reduced throughout oogenesis, but overall size of the egg chambers is not reduced. Therefore, the reduced oocyte size is not due to an overall growth defect in

<table>
<thead>
<tr>
<th>Stage</th>
<th>Control Total area*</th>
<th>Oocyte area</th>
<th>Control Ratio**</th>
<th>Bic-Dmom Total area*</th>
<th>Oocyte area</th>
<th>Bic-Dmom Ratio**</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-6</td>
<td>4110</td>
<td>671</td>
<td>0.16±0.01</td>
<td>5044</td>
<td>316</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>7</td>
<td>6227</td>
<td>1026</td>
<td>0.13±0.03</td>
<td>7435</td>
<td>510</td>
<td>0.08±0.01</td>
</tr>
<tr>
<td>8</td>
<td>13546</td>
<td>3081</td>
<td>0.21±0.05</td>
<td>13630</td>
<td>1959</td>
<td>0.12±0.05</td>
</tr>
<tr>
<td>9</td>
<td>26110</td>
<td>8901</td>
<td>0.34±0.01</td>
<td>30368</td>
<td>9102</td>
<td>0.30±0.02</td>
</tr>
<tr>
<td>10A</td>
<td>42849</td>
<td>15804</td>
<td>0.37±0.03</td>
<td>41888</td>
<td>14622</td>
<td>0.35±0.02</td>
</tr>
</tbody>
</table>

*Area of oocyte + nurse cells.

**The ratio of oocyte area over total area was determined for each egg chamber of a given stage and then the average of these ratios was calculated.
the 16-cell germarial cyst or to failure in yolk uptake, but instead is most likely due to reduced transfer of nurse cell contents into the oocyte. The effect on oocyte growth is instead most likely due to reduced transfer of nurse cell mRNAs into the oocyte. The effect on oocyte growth is therefore, implicates nurse-cell-to-oocyte transport early in oogenesis and, these defects may reflect a specific requirement for Bic-D activity present in these early stages (Table 2). We do not know whether the observed early localization of these mRNAs indicates that their localization is Bic-D independent or whether residual Bic-D activity present in these early stages is sufficient for their localization. It is conceivable that the latter may be the case because these egg chambers have sufficient Bic-D activity to make an oocyte and, as shown previously (Ran et al., 1994), residual Bic-D activity can be sufficient to localize certain mRNAs to the presumptive site of osk mRNA accumulation.

### Table 2. Localization of oocyte specific factors in Bic-Dmom and control egg chambers

<table>
<thead>
<tr>
<th>Localized factor</th>
<th>Genotype</th>
<th>Oogenesis stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2-6</td>
</tr>
<tr>
<td>gurken mRNA</td>
<td>wild-type</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>Bic-Dmom</td>
<td>78</td>
</tr>
<tr>
<td>fs(1)K10 mRNA</td>
<td>wild-type</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Bic-Dmom</td>
<td>95</td>
</tr>
<tr>
<td>oskar mRNA</td>
<td>wild-type</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Bic-Dmom</td>
<td>69</td>
</tr>
<tr>
<td>kinesin-β-gal*</td>
<td>wild-type</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Bic-Dmom</td>
<td>40</td>
</tr>
</tbody>
</table>

Numbers in bold represent the percentage of egg chambers in which the localized factor is detected at its usual site of accumulation: grk RNA, anterior oocyte in stage 7, perinuclear in stages 8-10A; fs(1)K10 (and orb), oocyte in stages 2-6, anterior oocyte in stages 7-10B; osk, oocyte in stages 2-6, anterior in stages 7-8, posterior in stages 8-10B; kinesin-β-gal, posterior in stages 8-10A. Numbers in parentheses represent total number of egg chambers examined for each stage.

*Localization of the kinesin-β-gal fusion protein as visualized by β-gal staining.
In wild-type oogenesis, osk mRNA appears transiently at the anterior of the oocyte in stage 8, and then starts to accumulate at the posterior where it stays for the remainder of oogenesis. In ovaries from females carrying Bic-D dominant mutations, some osk mRNA is retained at the anterior of the oocyte late in oogenesis, while the posterior accumulation of osk is not noticeably affected (Ephrussi et al., 1991; Kim-Ha et al., 1991). To determine if loss of Bic-D activity in later oogenesis also affects osk mRNA localization, we examined the distribution of osk transcripts in stage 8 to 10B Bic-Dmom egg chambers. The transient anterior accumulation and early posterior accumulation of osk transcript is normal, but in stages 9 and 10, the posterior accumulation becomes progressively reduced and often no localized transcript is detected (Table 2; Fig. 5B). This effect on osk is clearly distinct from that exerted by dominant Bic-D mutations, indicating that the Bic-D dominant alleles are neomorphs with respect to their effect on osk localization. Because eggs with a high expressivity of the Bic-Dmom phenotype are not fertilized, we have not been able to determine if the failure to maintain osk localization in mutant egg chambers results in production of embryos with abdominal defects.

To determine whether Bic-D is required specifically for the localization of osk mRNA or more generally for RNA localization within the oocyte, we examined the distribution of two other localized transcripts, orb and fs(1)K10 in late stage Bic-Dmom egg chambers. These mRNAs normally accumulate at the anterior margin in stage 8 where they remain until late in stage 10 (Lantz et al., 1992; Cheung et al., 1992). In Bic-Dmom egg chambers, both of these transcripts initially accumulate at the anterior in stage 8 but, in later stages, the amount of localized transcript is reduced or undetectable (Table 2, Fig. 5).

**Bic-D requirement in localizing patterning factors within the oocyte**

In wild-type oogenesis, osk mRNA appears transiently at the anterior of the oocyte in stage 8, and then starts to accumulate at the posterior where it stays for the remainder of oogenesis. In ovaries from females carrying Bic-D dominant mutations, some osk mRNA is retained at the anterior of the oocyte late in oogenesis, while the posterior accumulation of osk is not noticeably affected (Ephrussi et al., 1991; Kim-Ha et al., 1991). To determine if loss of Bic-D activity in later oogenesis also affects osk mRNA localization, we examined the distribution of osk transcripts in stage 8 to 10B Bic-Dmom egg chambers. The transient anterior accumulation and early posterior accumulation of osk transcript is normal, but in stages 9 and 10, the posterior accumulation becomes progressively reduced and often no localized transcript is detected (Table 2; Fig. 5B). This effect on osk is clearly distinct from that exerted by dominant Bic-D mutations, indicating that the Bic-D dominant alleles are neomorphs with respect to their effect on osk localization. Because eggs with a high expressivity of the Bic-Dmom phenotype are not fertilized, we have not been able to determine if the failure to maintain osk localization in mutant egg chambers results in production of embryos with abdominal defects.

To determine whether Bic-D is required specifically for the localization of osk mRNA or more generally for RNA localization within the oocyte, we examined the distribution of two other localized transcripts, orb and fs(1)K10 in late stage Bic-Dmom egg chambers. These mRNAs normally accumulate at the anterior margin in stage 8 where they remain until late in stage 10 (Lantz et al., 1992; Cheung et al., 1992). In Bic-Dmom egg chambers, both of these transcripts initially accumulate at the anterior in stage 8 but, in later stages, the amount of localized transcript is reduced or undetectable (Table 2, Fig. 5).

**Bic-D requirement in localizing patterning factors within the oocyte**

In wild-type oogenesis, osk mRNA appears transiently at the anterior of the oocyte in stage 8, and then starts to accumulate at the posterior where it stays for the remainder of oogenesis. In ovaries from females carrying Bic-D dominant mutations, some osk mRNA is retained at the anterior of the oocyte late in oogenesis, while the posterior accumulation of osk is not noticeably affected (Ephrussi et al., 1991; Kim-Ha et al., 1991). To determine if loss of Bic-D activity in later oogenesis also affects osk mRNA localization, we examined the distribution of osk transcripts in stage 8 to 10B Bic-Dmom egg chambers. The transient anterior accumulation and early posterior accumulation of osk transcript is normal, but in stages 9 and 10, the posterior accumulation becomes progressively reduced and often no localized transcript is detected (Table 2; Fig. 5B). This effect on osk is clearly distinct from that exerted by dominant Bic-D mutations, indicating that the Bic-D dominant alleles are neomorphs with respect to their effect on osk localization. Because eggs with a high expressivity of the Bic-Dmom phenotype are not fertilized, we have not been able to determine if the failure to maintain osk localization in mutant egg chambers results in production of embryos with abdominal defects.

To determine whether Bic-D is required specifically for the localization of osk mRNA or more generally for RNA localization within the oocyte, we examined the distribution of two other localized transcripts, orb and fs(1)K10 in late stage Bic-Dmom egg chambers. These mRNAs normally accumulate at the anterior margin in stage 8 where they remain until late in stage 10 (Lantz et al., 1992; Cheung et al., 1992). In Bic-Dmom egg chambers, both of these transcripts initially accumulate at the anterior in stage 8 but, in later stages, the amount of localized transcript is reduced or undetectable (Table 2, Fig. 5).
DISCUSSION

Bic-D role in dorsal/ventral patterning

In wild-type oogenesis, localized grk signal is thought to activate the top/EGF receptor in overlying columnar follicle cells, setting off a signal transduction cascade that leads to the establishment of dorsal/anterior follicle cell fate. This newly established fate is first seen in the expression of specific marker genes. Later in oogenesis, these cells secrete specialized dorsal/anterior chorionic structures, the dorsal appendages (Schüpbach and Roth, 1994).

In Bic-D

egg chambers, the posterior displacement of the oocyte and grk signal correlates with a posterior shift in expression of the dorsal/anterior columnar follicle cell marker BB142, and this also correlates with a posterior displacement of the dorsal appendages. However, the posteriorly displaced appendages are typically reduced and often no dorsal appendage material is formed at all (Fig. 2C,E). This is surprising given that BB142 expression, though displaced, reaches levels indistinguishable from those in wild type (Fig. 3F). While it may be that grk signaling to posterior follicle cells is in fact slightly reduced, it is also possible that the failure to properly make dorsal appendages is due to an inability of posterior follicle cells to adopt this fate in response to grk signaling. This latter possibility is supported by experiments in which an activated form of D-Raf is expressed in all follicle cells (Brand and Perrimon, 1994). Induction of this transgene results in the expression of the dorsal/anterior follicle cell marker AN296 in all follicle cells overlying the oocyte, but only those follicle cells in a ring at the anterior respond by secreting dorsal appendage material. These results, along with our own findings can be explained if anterior columnar follicle cells are fully competent to make dorsal appendages in response to grk signaling whereas more posterior follicle cells are not. This may imply that the columnar follicle cells are prepatterned along their anterior/posterior axis prior to reception of grk signal.

Autonomous patterning of follicle cell fates

Normally by stage 10 of oogenesis, follicle cell identities correspond tightly with the position of these cells relative to the underlying germ line. The anteriormost flattened follicle cells overlie the nurse cells. The remaining follicle cells make up a columnar epithelium, which precisely covers the oocyte. The most anterior of these columnar follicle cells overlie the nurse cell/oocyte boundary and later initiate centripetal migration at this site. Also, as described above, only the follicle cells overlying the anterior of the oocyte appear to be competent to respond properly to the anteriorly localized grk signal.

In Bic-D

even though the oocyte is smaller than in wild type, the position of the different follicle cell types is unaffected. Approximately the same number of follicle cells become columnar and migrate posteriorly. As a result, by stage 10A the most anterior situated of these come to overly nurse cells instead of the oocyte (Figs 3D, 4B). Therefore, follicle cell thickening and posterior migration are not dependent on the size of the oocyte. Centripetal cells are also determined independent of their position relative to the germ line. In Bic-D

egg chambers, these anterior columnar follicle cells still express the centripetal cell marker BB127, and usually initiate centripetal cell migration even though they do not overly the nurse cell/oocyte boundary (Fig. 4B,C). Therefore, there is evidence for a prepatternning of follicle cell fates along the A/P axis independent of their position relative to the underlying germ line.

Bic-D role in localizing factors within the egg chamber

Indirect evidence has pointed to a role for microtubule-based transport or anchoring in the transfer of nurse cell contents into the oocyte. Beginning in region 2b of the germarium, around the time that specific factors begin to accumulate in the oocyte, microtubules are arranged with minus ends in the oocyte and plus ends extending through ring canals into the other 15 nurse cells (Theurkauf et al., 1992). In egg chambers treated with microtubule-destabilizing drugs, the presumptive oocyte adopts a nurse cell fate (Koch and Spitzer, 1983) and oocyte-specific factors fail to accumulate in a single cell (Theurkauf et al., 1993). Recessive alleles of Bic-D result in a similar block in oocyte differentiation. In these mutant ovaries, failure to make an oocyte correlates with a failure to localize oocyte-specific mRNAs and proteins, and failure to form a visible microtubule organizing center in the presumptive oocyte (Suter and Steward, 1991; Theurkauf et al., 1993; Ran et al., 1994).

Bic-D could therefore be either a factor required in the pro-oocyte for differentiation of an oocyte, or a component of a transport or anchoring system that localizes such factors to the presumptive oocyte. Studying the precise role of Bic-D in oocyte determination is hampered by the small size of the germ line cells in these early stages and by the fact that lack of Bic-D causes the presumptive oocyte to adopt a nurse cell fate, inducing many cellular changes that do not directly depend on Bic-D. Analyzing the later requirements for Bic-D in oogenesis circumvents these problems and should thus allow us to test hypotheses for molecular roles of Bic-D.

From this analysis, we have found that Bic-D is required for...
oocyte growth in early oogenesis, corresponding to the stages when microtubules are asymmetrically arranged within the 16-cell cyst such that they could support directed transport into the oocyte. Treatment of wild-type egg chambers with microtubule-stabilizing drugs during these stages also leads to reduced oocyte growth (Koch and Spitzer, 1983), suggesting that both Bic-D and microtubules are involved in nurse-cell-to-oocyte transport early in oogenesis. These findings support the idea that the oocyte determination block in Bic-D mutants and in flies treated with microtubule-disrupting drugs is due primarily to a block in transport into the presumptive oocyte.

We have also found that Bic-D is required for the localization or maintenance of fs(1)K10 and orb transcripts at the anterior of the oocyte and osk mRNA at the posterior of the oocyte late in oogenesis (stages 9 to 10B, Fig. 5B,D, Table 2). Inhibitor studies have shown that transport and/or maintenance of these localized transcripts depends on microtubules (Pokrywka and Stephenson, 1991; Clark et al., 1994; Pokrywka and Stephenson, 1995). Bic-D is also required late in oogenesis to maintain the correct positioning of the oocyte nucleus (Fig. 4A), a process that is also dependent on microtubules (Koch and Spitzer, 1983). Taken together, these results suggest that Bic-D may be part of a microtubule-based transport or anchoring system that is utilized repeatedly throughout oogenesis.

One possible role for Bic-D in oocyte determination is in the establishment of the polarized microtubule network that connects the presumptive oocyte to the other 15 germ-line cells. If this is the case, the later requirements for Bic-D might reflect a similar role in organizing microtubules later in oogenesis. Three different results argue that this is not the case. First, using the kinesin-β-gal fusion protein as a marker for microtubule polarity, we find that, in Bic-D<sup>-dom</sup>, microtubules are organized correctly in stages 8 to 10B of oogenesis (Fig. 5F). Second, the microtubule-dependent rapid ooplasmic streaming, which begins in stage 10B, is not affected. Third, immunostaining of mutant egg chambers with an anti-tubulin antibody reveals no effects on microtubule organization throughout oogenesis (data not shown).

Our results favor a model in which Bic-D is involved in the transport or anchoring of localized factors along an existing microtubule network early and late in oogenesis. Bic-D does not seem to encode a microtubule-based motor (Suter et al., 1989; Wharton and Struhl, 1989), but it may act as an intermediate in either transport or anchoring of factors to microtubules. Such intermediate proteins have been described in other systems: the dynactin complex appears to mediate interactions between localized factors and microtubules (Schroer and Sheetz, 1991). By analogy, similar proteins would be expected to mediate the interactions between localized factors and microtubules in the Drosophila egg chamber. It is not yet possible to distinguish between a transport or anchoring role for Bic-D. One way to distinguish between these models will be to identify the factors that interact with Bic-D and to follow their movement in wild type and in Bic-D<sup>-dom</sup> egg chambers.

We wish to thank Thuy Nguyen, Akira Nakamura and Stéphane Larochelle for critical reading of the manuscript. Thanks to Trudi Schüpbach for providing us with gurken clones and alleles and enhancer trap lines. Thanks to Paul Schnell for the pElba plasmid and to Ira Clark for the kinesin-lacZ stock. This research is supported by the National Sciences and Engineering Research Council of Canada and by the National Cancer Institute of Canada with funds from the Canadian Cancer Society and the Terry Fox Run. B. S. is a Research Scientist of the National Cancer Institute of Canada supported by funds from the Canadian Cancer Society.

REFERENCES


Bic-D in mid-oogenesis 3585


(Accepted 31 July 1996)