Multiple roles for Egalitarian in polarization of the *Drosophila* egg chamber


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Running title: The multiple functions of Egalitarian
Abstract
The *Drosophila* egg chamber provides a useful model for examining mechanisms by which cell fates are specified and maintained in the context of a complex tissue. The egg chamber is also an excellent model for understanding the mechanism by which cytoskeletal filaments are organized, and the critical interplay between cytoskeletal organization, polarity establishment, and cell fate specification. Previous work has shown that Egalitarian (Egl) is required for specification and maintenance of oocyte fate. Mutants in *egl* either completely fail to specify an oocyte, or if specified, the oocyte eventually reverts back to nurse cell fate. Due to this very early role for Egl in egg chamber maturation, it is unclear whether later stages of egg chamber development also require Egl function. In this report, we have depleted Egl at specific stages of egg chamber development. We demonstrate that in early stage egg chambers, Egl has an additional role in organization of oocyte microtubules. In the absence of Egl function, oocyte microtubules completely fail to reorganize. As such, the localization of microtubule motors and their cargo is disrupted. In addition, Egl also appears to function in regulating the translation of critical polarity determining mRNAs. Finally, we demonstrate that in mid stage egg chambers, Egl does not appear to be required for microtubule organization, but rather for the correct spatial localization of *oskar*, *bicoid* and *gurken* mRNAs.
Introduction
The proper formation of a tissue or organ requires the precise specification of cell fates and the subsequent maintenance of these differentiated fates. Often, these processes take place during embryonic development. In many instances, disruption of tissue morphogenesis results in organismal lethality, complicating mechanistic studies. In this regard, the *Drosophila melanogaster* egg chamber is a useful tool for study. The ovary is a non-essential organ. Thus, genes with essential roles in formation of the mature *Drosophila* egg can be studied using adult animals. In addition, abundant genetic and molecular tools are available in *Drosophila*, facilitating mechanistic analysis of tissue morphogenesis (Sullivan et al. 2000).

Each *Drosophila* ovary is composed of 16 to 20 ovarioles (Spradling 1993). The germline stem cells and their associated somatic niche are found at the anterior tip of the ovariole in a region known as the germarium (Spradling 1993) (Fig. 1A, B). Division of a germline stem cell produces a daughter cell known as a cystoblast (Spradling et al. 2001; Gonzalez-Reyes 2003). The cystoblast undergoes four rounds of cell division to produce a cyst containing 16 germ cells. The cyst is eventually surrounded by a layer of somatic cells known as follicle cells. As the cyst progresses through the germarium, oocyte fate is specified (Deng and Lin 2001; Riechmann and Ephrussi 2001; Huynh and St Johnston 2004). Thus, once the cyst emerges from the germarium as an egg chamber, it contains 15 nurse cells and 1 oocyte (Spradling 1993)(Fig. 1A, B). The egg chamber progresses through 14 stages of morphogenesis before it is competent for fertilization. During these stages, the cyst grows in size and distinct fates are specified in the follicle cells (Spradling 1993). Although 14 different stages of egg chamber maturation can be identified based on morphological features and the cell fates that are specified, egg chamber maturation represents a developmental continuum. Thus, not every stage of egg chamber development is observed in all ovarioles.

Between stages 2 and 6 there is a build up of gurken (grk) mRNA and protein within the oocyte (Neuman-Silberberg and Schupbach 1993; Neuman-Silberberg and Schupbach 1996). This pool of Grk signals to the overlying follicle cells to adopt posterior cell fates (Gonzalez-Reyes et al. 1995; Roth et al. 1995). Once posterior fate has been specified, the follicle cells signal back to the oocyte. Although the nature of this signal is still unknown, the Notch and Hippo pathways are thought to contribute to the process (Ruohola et al. 1991; Meignin et al. 2007; Polesello and Tapon 2007; Yu et al. 2008; Yan et al. 2011). In addition, proteins within the extracellular matrix and factors that link the oocyte to the follicle cells are also involved (Deng and Ruohola-Baker 2000; Frydman and Spradling 2001; MacDougall et al. 2001). This signaling cascade initiates a reorganization of oocyte microtubules. Prior to this, oocyte microtubules are arranged with minus ends enriched at the posterior pole (Theurkauf et al. 1992). However, the redistribution of microtubules that takes place in response to Grk signaling results in minus ends enriched along the anterior and lateral cortex, and plus ends that display a slight enrichment at the posterior pole of the oocyte (Theurkauf et al. 1992; Clark et al. 1994; Clark et al. 1997; Parton et al. 2011; Sanghavi et al. 2012). This reorganization is required for migration of the oocyte nucleus from the posterior to the anterior of the oocyte (Gonzalez-Reyes et al. 1995; Roth et al. 1995) (Fig. 1A). In addition, this microtubule organization is required for the posterior localization of oskar (osk) mRNA, the anterior localization of bicoid (bcd) mRNA, and the localization of grk mRNA at the dorsal-anterior margin (St Johnston 2005). The precise localization of these mRNAs and their resulting protein products is essential for polarizing the oocyte and the resulting embryo.
Given the complex patterning events that are required for producing a mature *Drosophila* egg, it is not surprising that many genes have been implicated in these various processes. Among these genes is *egalitarian* (*egl*). *egl* null mutants fail to specify an oocyte (Theurkauf *et al.* 1993; Carpenter 1994). Instead, egg chambers in these mutants contain 16 nurse cells (Theurkauf *et al.* 1993; Carpenter 1994). Egl is thought to function in this pathway by restricting meiosis to a single cell and by promoting the microtubule-dependent transport of factors to the presumptive oocyte (Mach and Lehmann 1997; Huynh and St Johnston 2000; Bolivar *et al.* 2001). Milder mutants in *egl* that are defective in binding Dynein light chain (Dlc), initially specify an oocyte. However, oocyte fate is not maintained in these mutants, eventually resulting in cysts containing 16 nurse cells (Navarro *et al.* 2004). In embryos, Egl has been shown to function as an adaptor for Dynein, by linking this microtubule motor to mRNAs destined for localization (Dienstbier *et al.* 2009). Thus, Egl is required at very early stages of *Drosophila* egg chamber morphogenesis, and much later, during development of the embryo.

The role of Egl in oocyte specification and maintenance has precluded a detailed analysis of Egl function during mid and late stages of egg chamber development. Using an shRNA-mediated depletion strategy, we have been able to specifically deplete Egl at defined stages of egg chamber development. We demonstrate that in early stage egg chambers, Egl is required for organization of oocyte microtubules and translational regulation of specific mRNAs. In mid and late stage egg chambers, Egl does not appear to play a role in microtubule organization, but rather, functions in the localization of *osk, bcd,* and *grk* mRNAs. Thus, Egl function is required at multiple stages in the development of a mature *Drosophila* egg.
Materials and Methods

Fly stocks
The following fly stocks were used: Oregon-R-C (used as wild-type; Bloomington stock center; #5); GFP-Stau (Zimyanin et al. 2008); eb1 shRNA (Bloomington stock center; #36680, donor TRiP); egl shRNA-1 (Bloomington stock center; #43550, donor TRiP); khc shRNA (Bloomington stock center; #35409, donor TRiP); osk^84 (Kim-Ha et al. 1991); Df(3R)p-XT103 (Deficiency for osk, Bloomington stock center; #1962; donor Thom Kaufman); P[w+[mC]=UASp-Act5C.mRFP]13, w[+]; (Bloomington stock center; #24777, donor Susan Parkhurst); pnt-LacZ (Becalska and Gavis 2010), Par1GFP N1S isoform (Doerflinger et al. 2006); and Dp(2;3)BAC.cnn^eGFP::Ex1A (Eisman et al. 2015).

The expression of the shRNA and the rescue transgene was driven using either: P{w+[mC]=matalpha4-GAL-VP16}V37 (Referred to in the results section as the early-stage driver; Bloomington stock center; #7063, donor Andrea Brand) or w[+]; P{w+[mC]=matalpha4-GAL-VP16}V2H (Referred to in the results section as the mid-stage driver; Bloomington stock center; #7062, donor Andrea Brand).

The eglR-GFP transgene was constructed by first cloning the coding sequence of egl into pUC19. Site directed mutagenesis was used to mutate the following nucleotides within egl: A1599C and G1602C. This was done using the Q5 Site Directed Mutagenesis Kit (New England Biolabs). Once the desired mutations were confirmed by sequencing, the entire coding sequence of eglR was cloned into pUASp-attB-K10 (Koch et al. 2009). The EGFP coding sequence was sub-cloned immediately downstream of eglR. This resulted in the eglR-GFP transgene that encoded wild-type Egl protein, yet was not targeted by egl shRNA-1. Transgenic flies were generated by BestGene Inc. In order to test for rescue, crosses were set up to recombine eglR-GFP and egl shRNA-1 onto the same chromosome.

Egl shRNA-2 flies were generated by cloning the following sequence into the Nhel and EcoR1 sites of the Valium 22 vector (Ni et al. 2011): 5’-ACCGAGGTGTCCGATCTCAAA-3’. The insert was confirmed by sequencing. Transgenic flies contain this shRNA construct were generated by BestGene Inc. The egl shRNA-2 construct was inserted at the attP40 site on Chromosome 2.

Antibodies
Unless specifically stated, the indicated dilutions are for immunofluorescence. The following antibodies were used: rabbit anti-Egl (1:2000); rabbit anti-Staufen (D. St Johnston; 1:2500); mouse anti-Dhc (Developmental studies hybridoma bank; 1:150; donor J. Scholey); rabbit anti-Khc (Cytoskeleton, Inc., 1:150); mouse anti-BicD (Developmental studies hybridoma bank; 1:150; donor Ruth Steward); chicken anti-Oskar (1: 50 after blocking with osk protein null ovaries); mouse anti-Oskar (D. Chen, 1:1000); rabbit anti-Oskar (A. Ephrussi, 1: 2000); FITC conjugated mouse anti-alpha tubulin (Sigma; 1: 100); rabbit anti-Eb1 (S. Rogers, 1:500); mouse anti-β galactosidase (Promega; 1:2000); mouse anti-gamma-tubulin (Sigma; 1:100); mouse anti-Grk (Developmental Studies Hybridoma Bank, 1:175, donor T.
Schüpbach); rat anti-GFP (Nacalai USA, Inc.; 1:600); mouse anti-Orb (Developmental studies hybridoma bank; 1:300; donor P. Schedl); mouse anti-LaminDmO (Developmental studies hybridoma bank; clone ADL84.12; 1:200; donor P.A. Fisher); goat anti-rabbit Alexa 594 and 488 (Life technologies, 1:400 and 1:200 respectively); goat anti- mouse Alexa 594 and 488 (Life technologies, 1:400 and 1:200); goat anti-chicken 594 and 488 (Life technologies, 1:400 and 1:200 respectively).

The chicken anti-Osk antibody was prepared by injecting the following peptide into chicken (Aves Labs) : ESNYISVREEYPDIDSEVR. Yolk was collected and antibody specific to this peptide was purified by Aves Labs.

**Immunofluorescence**

Prior to dissection, flies were fattened on yeast paste for 3 days. Ovaries were dissected as previously described (Liu et al. 2015) and were fixed in either 8% formaldehyde for 10 minutes (for the alpha-tubulin staining experiment) or 4% formaldehyde for 20 minutes (for the remaining experiments). The fixative was diluted in PBS. For the experiments involving mouse and rabbit antibodies, the immunofluorescence staining was performed as previously described (Liu et al. 2015). For experiments involving the chicken anti-Osk antibody, the oocytes were blocked in BlokHen II™ (Aves labs) diluted 1:10 in PBS for 30 mins. The primary antibody was incubated in 1xPBST (PBS + 0.1% triton X-100) + 0.2% BSA overnight at 4°C. The next day, the samples were washed three times in PBST. The ovaries were then incubated in goat anti-Chicken Alexa 594 (Life technologies). The secondary antibody was diluted in 1xPBST + 0.2% BSA and incubated overnight at 4°C. The following day, the ovaries were washed three times in PBST, mounted on slides and imaged.

**In situ hybridization**

 osk, bcd, and grk mRNAs were visualized as previously described (Sanghavi et al. 2013).

**Microscopy**

Images were captured on a Zeiss LSM 780 upright or a Zeiss LSM 510 upright confocal microscope. Images were processed for presentation using Fiji, Adobe Photoshop and Adobe Illustrator.

**Quantification**

Localization phenotypes were quantified by scoring egg chambers of the indicated genotype from at least three different experiments. The level of Egl in stage 10 egg chamber (Fig. 2E) was quantified using the Zen software provided by Zeiss. The level of fluorescence intensity in a 50 micron-square area in the nurse cells of control and Egl depleted egg chambers was determined. This was done on a single confocal slice of 25 different egg chambers for each genotype. The level of Grk oocyte enrichment (Fig. 7E and Supplemental Fig. 5E) was determined using the same software. For this analysis, the fluorescence intensity within the oocyte was compared to the fluorescence intensity within the remainder of the egg chamber. A single confocal slice in the middle plane of the egg chamber was used for this
analysis. For these experiments, 15 egg chambers from each genotype were quantified. The statistical calculators provided by Graph pad were used for calculating significance. An unpaired t test was performed using Standard deviation, mean and the n value.

**Analyzing mRNA association.**
Ovaries were dissected from flies expressing GFP or Egl-GFP. 600 micro-gram of each lysates were then incubated with GFP-Trap beads (Chromotek) in order to purify GFP and Egl-GFP. The procedure for immunoprecipitation followed by RT-PCR was previously described (Sanghavi et al. 2013). The RNA that co-precipitated with GFP and Egl-GFP was reverse transcribed using random hexamers and Superscript III (Life technologies) using the directions provided by the manufacturer. The concentration of the cDNA was determined and 60 ng of cDNA was used in each quantitative PCR reaction. The SsoAdvanced Universal SYBR Green Supermix from BioRad was used for qPCR. The reaction was run using a Bio-Rad CFX96 Real-time PCR System. The following primers were used in the qPCR reactions:

1. osk mRNA
   - Forward: 5’-CCCGAGGGTACTCAGATCAT-3’
   - Reverse: 5’-GCGGTGCAAGATTTGTTAGA-3’

2. bcd mRNA
   - Forward: 5’-ATCCGGATCGCACAAGGAC-3’
   - Reverse: 5’-GCCTTAATGACTCGCTGTA-3’

3. grk mRNA
   - Forward: 5’-ATCCGGATCGCACAAGGAC-3’
   - Reverse: 5’-CGACGACAGCAGAGGAGTA-3’

4. rp49 mRNA
   - Forward: 5’-CCAGTCGGATCTGCTGTA-3’
   - Reverse: 5’-GGGCATCAGATCTGCTGTA-3’

5. gamma-tubulin mRNA
   - Forward: 5’-CCACCATCAGATCTGAGC-3’
   - Reverse: 5’-ACCGATGAGTGGTTGTTCCA-3’

In order to determine mRNA association, the ct values were compared to gamma-tubulin. rp49 represents an un-localized mRNA and serves as a further control. The entire experiment was done in triplicate.

**Analyzing mRNA levels.**
Ovaries were dissected from flies expressing a control shRNA against eb1, or from flies expressing egl shRNA-1. RNA was extracted using TRizol (Life technologies) following the directions provided by the manufacturer. cDNA was generated using 5 micro-gram of total RNA from each sample. Random hexamers and Superscript III (Life technologies) was used for the reverse transcription. 15ng of cDNA was used in each qPCR reaction. The primer sequences are shown above. The geometric mean was determined for gamma-tubulin and rp49 and this was used to compare to the levels of osk, bcd and grk. The entire experiment was done in triplicate.

**Data availability.**
All strains used in this work will be made available upon request.
Results
The localization and RNA binding properties of Egalitarian.
Egalitarian (Egl) is a Dynein adaptor that has been shown to link this motor with localized mRNAs in the Drosophila embryo (Navarro et al. 2004; Dienstbier et al. 2009). Within the ovary, the majority of studies have focused on the role of Egl in specification and maintenance of oocyte fate (Theurkauf et al. 1993; Mach and Lehmann 1997; Huynh and St Johnston 2000; Bolivar et al. 2001). The goal of the present study was to examine the function of Egl during mid-stages of egg chamber maturation.

Dynein heavy chain (Dhc), the motor subunit of the Dynein complex, was enriched at the posterior pole of the oocyte in stage 10 egg chambers (Fig. 1C, arrow). This localization pattern is consistent with published findings (McGrail et al. 1995). Partial co-localization was observed between Egl and Dhc at the posterior pole and along the oocyte cortex (Fig. 1C). It should be noted, however, that whereas Dhc displayed an obvious enrichment at the posterior pole, the posterior enrichment of Egl was slightly less apparent (Fig. 1C).

We next determined whether Egl preferentially associated with mRNAs destined for localization within the oocyte. Although numerous mRNAs are specifically localized within the Drosophila egg chamber (Jambor et al. 2015), the localization pathways of gurken (grk), bicoid (bcd), and oskar (osk) mRNAs have been most extensively characterized (Gonsalvez and Long 2012; Weil 2014). Lysates expressing GFP or Egl-GFP were subjected to immuno-precipitation using GFP antibody beads. The co-precipitating mRNAs were extracted and analyzed using reverse transcriptase (RT) followed by quantitative PCR. The values were normalized to the level of gamma-tubulin mRNA detected in each pellet. The non-localizing mRNA, rp49, was not specifically enriched in the Egl pellet (Fig. 1D). By contrast, a significant enrichment of bcd mRNA was detected in the Egl pellet (Fig. 1D). grk mRNA, and to a lesser extent, osk mRNA, were also enriched in Egl pellets (Fig. 1D). Thus, as observed in Drosophila embryos (Dienstbier et al. 2009; Vazquez-Pianzola et al. 2014), Egl associates with mRNAs that are destined for localization.

Depletion of Egl in mid-stage egg chambers.
Classical mutant alleles of egl have not been very useful in examining the role of this protein during mid-stages of egg chamber maturation. Egl performs an essential function in oocyte specification. Consequently, loss-of-function mutants in egl never specify an oocyte (Theurkauf et al. 1993; Carpenter 1994). Mutants in egl that are defective in binding Dynein light chain (Dlc) initially specify an oocyte. However, oocyte fate is not maintained in these mutants, and the oocyte eventually reverts to a nurse cell fate (Navarro et al. 2004). Occasionally, these mutants produce morphologically normal stage 10 egg chambers, and within these, osk mRNA is often delocalized (Navarro et al. 2004). These results suggest an important role for Egl in localizing mRNAs in mid-stage egg chambers.

In order to more directly examine the role of Egl in mRNA localization, we employed an shRNA-mediated depletion strategy. We have successfully used this approach to analyze the role of Dhc and the Dynein regulator, Dynamitin, in mRNA localization and endocytosis in mid-stage egg chambers (Sanghavi et al. 2013; Liu et al. 2015). A strain expressing an shRNA against egl (egl shRNA-1) was obtained from the Bloomington Drosophila stock center. In addition, we generated an independent shRNA targeting a different region of egl (egl shRNA-2) (materials and methods for details). The shRNAs were expressed using a maternal alpha-tubulin driver that is only active after the oocyte has been specified
(Supplemental Fig. 1A). This expression profile enables us to bypass the requirement for Egl in oocyte specification, and to study the role of this protein during later stages of oogenesis.

We next determined the efficacy and specificity of Egl depletion using fly strains expressing a control shRNA, egl shRNA-1 or egl shRNA-2. Egg chambers from these flies were fixed and processed for immunofluorescence using an antibody against Egl. In control flies, Egl was abundantly expressed in the germline and soma during all stages of oogenesis (Fig. 2A, C). By contrast, Egl was significantly depleted from the germline of flies expressing egl shRNA-1 (Fig. 2B, D). Typically, depletion was observed from stage 5 onwards (Fig. 2B, D). Consistent with the expression profile of the driver, the level of Egl in the somatic follicle cells was unaffected in these flies (Fig. 2D, arrow, arrowhead). Quantification of Egl depletion in stage 10 egg chambers is shown in Fig. 2E. Similar results were obtained by western blot analysis of whole ovary lysates (Supplemental Fig. 2A). It should be noted however, that this analysis under-represents the germline specific depletion of Egl. These lysates contain follicle cells and stage 1 through 4 egg chambers. As shown by immunofluorescence analysis, Egl is not depleted in somatic cells and in these very early stage egg chambers (Fig. 2B). In contrast to egl shRNA-1, expression of egl shRNA-2 was not capable of depleting endogenous Egl (Supplemental Fig. 2B). Therefore, most of the remaining experiments were carried out using flies expressing egl shRNA-1.

**Egl is required for localization of osk, grk and bcd mRNAs.**

osk mRNA localizes to the posterior pole of the oocyte in wild-type stage 10 egg chambers (Ephrussi et al. 1991; Kim-Ha et al. 1991). The localization of osk mRNA plays an important role in restricting the expression of Oskar (Osk) protein to this site. The same posterior localization pattern was observed for osk mRNA in egg chambers expressing a control shRNA or egl shRNA-2 (Fig. 3A, arrow, data not shown). By contrast, osk mRNA was completely delocalized in egg chambers expressing egl shRNA-1 (Fig. 3B, L). The delocalized osk mRNA was not only present in the oocyte, but also in the nurse cell cytoplasm (Fig. 3B).

Imaging the delocalized osk mRNA required a higher gain setting than what was used for control egg chambers. This result is expected because in control egg chambers most molecules of osk mRNA are highly enriched at the posterior pole, confined to a relatively small three-dimensional volume. When osk mRNA is delocalized, the same number of osk molecules is now distributed over a much larger volume. Thus, visualizing the delocalized osk mRNA requires a higher gain. However, an alternative interpretation of this result is that the level of osk mRNA is reduced upon depletion of Egl. To address this latter possibility, we performed quantitative analysis using total RNA extracted from control and egl shRNA-1 expressing ovaries. This analysis revealed no statistical difference in the level of osk mRNA between these two strains (Supplemental Fig. 2C).

In order to verify that the diffuse signal corresponds to delocalized osk mRNA, these strains were processed for in situ hybridization using a sense probe against osk. The egg chambers were imaged using the same high-gain setting. Despite using 10 times more sense probe in comparison to anti-sense probe, we did not detect any signal in Egl depleted oocytes (Fig. 3C). Thus, the in situ hybridization signal is specific for delocalized osk mRNA. As a final test, control and Egl depleted egg chambers were processed for immunofluorescence using an antibody against Staufen, a core component of the osk mRNP (St Johnston et al. 1991; Zimyanin et al. 2008). Consistent with the in situ hybridization result, Staufen was absent from the oocyte posterior in Egl depleted oocyte (Fig. 3D, E). As expected, expression of egl
shRNA-2 had no effect on the localization of Staufen (Fig. 3F, arrow).

We next examined the localization of \textit{grk} and \textit{bcd} mRNAs. \textit{grk} mRNA localizes to the dorsal anterior corner of stage 10 wild-type egg chambers (Neuman-Silberberg and Schupbach 1993). This localization pattern is required for concentrating Gurken (Grk) protein at this site. In contrast to egg chambers expressing a control shRNA, \textit{grk} mRNA was completely delocalized in Egl depleted egg chambers (Fig. 3G, arrow, H, L). \textit{bcd} mRNA localizes to the anterior margin of stage 10 egg chambers (Berleth \textit{et al.} 1988). This results in a high concentration of \textit{bcd} mRNA at the anterior pole of wild-type embryos (Berleth \textit{et al.} 1988). As with \textit{osk} and \textit{grk} mRNAs, \textit{bcd} mRNA was completely delocalized in Egl depleted egg chambers (Fig. 3I-L). In approximately 45% of egg chambers, \textit{bcd} mRNA was diffusely localized throughout the entire egg chamber (Fig. 3J, L). In the remainder, \textit{bcd} mRNA was localized along the oocyte cortex (Fig. 3K, L). As noted with \textit{osk}, the level of \textit{grk} and \textit{bcd} mRNAs were unchanged upon depletion of Egl (Supplemental Fig. 2C).

A potential caveat of these results is that these phenotypes might be non-specific and caused due to off-target depletion of unknown factors. In order to address this issue, we attempted to rescue the mRNA localization defect using a wild-type Egl-GFP transgene. An shRNA resistant Egl transgene was generated by introducing mutations at the wobble position within the shRNA-1 recognition site of \textit{egl} (Supplemental Fig. 3A). The net result was an \textit{egl}R-GFP transgene that encoded wild-type Egl protein, yet was not targeted by the shRNA. This transgene was then introduced into the \textit{egl}shRNA-1 background. The expression of \textit{egl} shRNA-1 as well as transgenic EglR-GFP was under the control of the same driver. Thus, as the level of endogenous Egl is depleted, transgenic EglR-GFP is expressed. For simplicity, we will refer to these as “rescue” flies.

We first confirmed that EglR-GFP was expressed in the rescue flies and that it localized in a similar manner to endogenous Egl (Supplemental Fig. 3B). We next examined the localization of \textit{osk}, \textit{grk} and \textit{bcd} mRNAs. The localization pattern of all three mRNAs resembled wild-type (Supplemental Fig. 3C-F). Based on these results, we conclude that the mRNA delocalization phenotype is caused due to specific depletion of Egl.

**Egl regulates the translation of \textit{osk} and \textit{grk} mRNAs.**

In wild-type egg chambers, translation of \textit{osk} mRNA is repressed during its transport to the posterior pole (Kim-Ha \textit{et al.} 1995; Johnstone and Lasko 2001). Once \textit{osk} mRNA is correctly localized, translational repression is relieved, and the mRNA is activated for translation (Johnstone and Lasko 2001). Several factors have been identified that are required for precisely regulating the translation of \textit{osk} mRNA (Wilhelm and Smibert 2005). Osk protein performs an essential function in maintaining the anterior-posterior polarity of the oocyte and embryo (Ephrussi and Lehmann 1992; Smith \textit{et al.} 1992). Thus, ectopic expression of Osk results in oocytes with defective polarity, and embryos that are not able to establish proper cell fates. As shown in the preceding section, depletion of Egl results in complete delocalization of \textit{osk} mRNA. We therefore wished to determine whether this delocalized message was translated.

In order to examine the expression and localization of Osk protein, we developed a chicken anti-Osk antibody. As expected, this antibody recognized the posterior restricted Osk protein in wild-type egg chambers (Fig. 4A, arrow, Supplemental Fig. 2D). By contrast, the posterior crescent of Osk protein was not observed in \textit{osk} protein-null egg chambers (Fig. 4B). Thus, this antibody is capable of detecting endogenous Osk. It should be noted, however, that
variable non-specific staining of follicle cells was observed using this antibody. The follicle cell staining was seen in both wild-type and osk protein-null egg chambers (Fig. 4A, B).

Egg chambers from flies expressing a control shRNA or egl shRNA-1 were fixed and processed for immunofluorescence using this chicken anti-Osk antibody. Surprisingly, this analysis revealed that in the absence of Egl, the delocalized osk mRNA was translated into protein (Fig. 4C, Supplemental Fig. 2E). As a control for specificity, we examined egg chambers from flies expressing egl shRNA-2. As noted previously, this shRNA is not capable of depleting endogenous Egl (Supplemental Fig. 2B). Consequently, osk mRNA remains localized in this strain, and Osk protein is restricted to the posterior pole (Fig. 4D, data not shown).

In order to verify that our antibody was specifically detecting Osk protein, we repeated the experiment using two additional anti-Osk antibodies, one generated in mouse and another generated in rabbit (Markussen et al. 1995; Huang et al. 2014). The same result was obtained using both antibodies (Fig. 4E-H, Supplemental Fig. 2F, G). Based on these findings, we conclude that in the absence of Egl, the delocalized osk mRNA is no longer maintained in a translationally repressed state.

We next examined the expression and localization of Grk protein. The enrichment of grk mRNA at the dorsal-anterior corner of the oocyte contributes to the localization of Grk protein at this site. Polarized secretion of Grk, and its signaling to the overlying follicle cells, is necessary for establishing the dorsal-ventral axis of the oocyte (Neuman-Silberberg and Schupbach 1993). In contrast to egg chambers expressing a control shRNA, dorsal-anterior signal for Grk protein was not observed in Egl depleted oocytes (Fig. 4I, J). In fact, specific signal that corresponds to Grk protein was almost absent in Egl depleted egg chambers (Fig. 4J). The net result of this phenotype is that the overlying follicle cells do not receive a signal to establish dorsal cell fates. Consequently, most mature eggs from these strains display a ventralized phenotype reflected by a loss of dorsal appendages (97%, n = 65, data not shown).

In order to validate this result, we examined the localization and expression of Grk protein in Khc depleted egg chambers. In khc null oocytes, grk mRNA is delocalized and the delocalized mRNA is translated into protein (Brendza et al. 2002; Duncan and Warrior 2002; Januschke et al. 2002). Similar results were obtained in Khc depleted oocytes; grk mRNA was delocalized, and some of the delocalized mRNA was translated into protein (Supplemental Fig. 4C, D, Fig. 4K). Importantly, in contrast to grk mRNA, delocalized osk mRNA was not translated in Khc depleted oocytes (Supplemental Fig. 4A, B, Fig. 4L).

Based on these results we conclude that in the absence of Egl, delocalized osk mRNA is inappropriately translated into protein. By contrast, the delocalized grk mRNA present in these same oocytes, appears to be translated much less efficiently. These results reveal an important distinction in the translational regulation of osk and grk mRNAs.

As shown in the previous section, expression of an shRNA-refractory eglR-GFP transgene was able to rescue the osk and grk mRNA localization defects (Supplemental Fig. 3C, E). The same flies were also examined using anti-Osk and anti-Grk antibodies. This revealed that Osk protein was restricted to the posterior pole in the rescue flies, and Grk protein was restricted to the dorsal-anterior corner of the oocyte (Supplemental Fig. 3G and data not shown). Thus, expression of the shRNA-refractory eglR-GFP transgene was able to rescue
the localization and translational regulation of osk and grk mRNAs.

bcd mRNA is translated in the embryo by a mechanism that involves lengthening of the poly A tail (Salles et al. 1994). Thus, wild-type oocytes express very little Bicoid (Bcd) protein (Driever and Nusslein-Volhard 1988)(data not shown). A similar situation was observed in Egl depleted oocytes. The delocalized bcd mRNA was not precociously translated into protein in these egg chambers (data not shown).

Egl is required for the normal localization of Dhc, Khc and BicD.

Egl is considered an adaptor of the Dynein motor. Egl binds directly to Dlc, a component of the Dynein complex (Navarro et al. 2004), and in Drosophila embryos, Egl serves to link Dynein to mRNAs destined for localization (Dienstbier et al. 2009). We therefore examined the localization of Dynein in Egl depleted egg chambers using an antibody against Dhc. In stage 10 egg chambers expressing a control shRNA, Dhc was enriched at the posterior pole of the oocyte and also along the anterior and lateral cortex (Fig. 5A). A similar pattern was observed in wild-type egg chambers (data not shown) (McGrail et al. 1995). In the absence of Egl, Dhc was no longer enriched at the oocyte posterior or along the cortex in stage 10 egg chambers. Rather, Dhc was diffusely distributed throughout the cytoplasm of the oocyte (Fig. 5B). A similar distribution was observed for Khc (Fig. 5A’, A”, B’, B”).

We next examined the localization of Bicaudal-D (BicD), an interacting partner of Egl (Mach and Lehmann 1997). In wild-type stage 10 egg chambers, and in strains expressing a control shRNA, BicD localized along the oocyte cortex (Fig. 5C, data not shown). As with Dynein and Kinesin, loss of Egl resulted in diffuse distribution of BicD throughout the oocyte with no obvious enrichment along the cortex (Fig. 5D).

Disruption of the Dynein motor is associated with defects in the localization of the oocyte nucleus (Swan et al. 1999; Brendza et al. 2002; Duncan and Warrior 2002; Januschke et al. 2002). In stage 10 wild-type egg chambers, or in egg chambers expressing a control shRNA, the oocyte nucleus was anchored at the dorsal-anterior corner of the oocyte (Fig. 5E, arrow, data not shown). By contrast, 48% of Egl depleted egg chambers contained a mis-localized oocyte nucleus. In some egg chambers, the oocyte nucleus was present close to the anterior, but was not anchored adjacent to the cortex (Fig. 5F, arrow). In addition, in several Egl depleted egg chambers, the oocyte nucleus was observed at the posterior pole, having completely failed to migrate towards the anterior (Fig. 5G, arrow). Thus, depletion of Egl disrupts the localization, and possibly the activity, of the Dynein motor complex. The normal localization of Dhc, Khc, BicD, and the oocyte nucleus was restored upon expression of the shRNA-refractory eglR-GFP transgene (Supplemental fig. 3H, I, and data not shown).

Microtubule organization is disrupted upon depletion of Egl.

The severe delocalization phenotype observed for Dhc, Khc and BicD upon depletion of Egl suggests that a defect in microtubule organization might underlie these phenotypes. In order to directly test this possibility, we first examined the overall organization of microtubules using an antibody against alpha-tubulin, a core component of the microtubule polymer. Stage 10 control egg chambers contained a dense array of microtubules along the anterior portion of the oocyte (Fig. 6A, arrow), whereas the posterior was characterized by an overall lower density of microtubules (Fig. 6A, asterisk). By contrast, a uniformly high level of alpha-tubulin staining was observed throughout the entire oocyte in Egl depleted egg chambers (Fig. 6B, C). Thus, depletion of Egl disrupts the normal organization of oocyte microtubules.
In order to more precisely define the organization of microtubules in Egl depleted oocytes, we examined these egg chambers using markers for microtubule plus-ends. The first marker used was an antibody against End binding protein1 (Eb1), a highly conserved plus-end binding protein (Schuyler and Pellman 2001). In wild-type egg chambers, Eb1 foci were detected throughout the oocyte, with enrichment at the posterior pole (Sanghavi et al. 2012) (Fig. 6D). In Egl depleted egg chambers, Eb1 foci were scattered throughout the oocyte with no obvious enrichment at the posterior pole (Fig. 6E). Occasionally, a cloud of Eb1 foci could be detected close to the posterior (Fig. 6E’).

We validated these results using another plus-end marker, a strain expressing the Kin:βgal transgene. This transgene represents a fusion between the motor domain of Khc and β-galactosidase (Clark et al. 1994). Kin:βgal localizes to the posterior pole of stage 9 and 10a egg chambers (Clark et al. 1994) (Fig. 6F, arrow). This was not observed in Egl depleted oocytes. Instead, diffuse signal for Kin:βgal was observed throughout the oocyte (Fig 6G). Thus, upon depletion of Egl, microtubule plus-ends, which are normally enriched at the posterior pole, become scattered within the oocyte.

We next examined the localization of microtubule minus-ends. An antibody against gamma-tubulin, a core component of microtubule organizing centers, was used for this analysis. Gamma-tubulin was enriched along the oocyte cortex in stage 9 and 10 control egg chambers (Fig. 6H) (Cha et al. 2002). The interior of control oocytes was characterized by a lower level of gamma-tubulin staining (Fig. 6H) (Cha et al. 2002). In Egl depleted oocytes, the cortical enrichment of gamma-tubulin was not detected. Instead, signal for gamma-tubulin was detected throughout the entire oocyte (Fig. 6I). The normal distribution of microtubule plus and minus-ends was restored upon expression of the shRNA-refractory eglR-GFP transgene (Supplemental Fig. 3J-L), thus demonstrating the specificity of these phenotypes.

During the course of these experiments, we noticed a stage-specific redistribution of gamma-tubulin in control egg chambers. In early stage egg chambers, gamma-tubulin was uniformly distributed throughout the entire egg chamber (Fig. 6J, white arrow). At stage 7 there was a transition in the localization of gamma-tubulin within the oocyte. From stage 7 onwards, gamma-tubulin became concentrated along the oocyte cortex, and very little signal was observed within the oocyte cytoplasm (Fig. 6J, red arrow, L). In contrast to the control, this transition did not occur in Egl depleted egg chambers, and gamma-tubulin remained diffusely distributed (Fig. 6K, M). Thus, Egl appears to be required for this oocyte-specific reorganization of microtubule minus-ends.

Similar results were obtained using a strain expressing GFP-tagged Centrosomin (Cnn), another component of microtubule organizing centers (Megraw et al. 1999). In control egg chambers, GFP-Cnn was enriched around the cortex of the oocyte (Fig. 6N). This pattern was disrupted upon depletion of Egl. In 68% of egg chambers, GFP-Cnn was diffusely distributed throughout the entire oocyte (Fig. 6O). In the remaining egg chambers, GFP-Cnn signal was still detected within the interior of Egl depleted oocytes. However, residual cortical enrichment of GFP-Cnn could also be detected (Fig. 6P, arrow).

The role of Egl in microtubule organization.
The reorganization of oocyte microtubules that occurs in stage 7 egg chambers is initiated by Grk protein. During early stages of egg chamber maturation, grk mRNA and protein become selectively enriched within the oocyte (Neuman-Silberberg and Schupbach 1996).
The oocyte-enriched Grk protein signals the overlying follicle cells to establish posterior cell fate (Gonzalez-Reyes et al. 1995; Roth et al. 1995). Once established, posterior follicle cells signal back to the oocyte by a mechanism that remains poorly understood. The net result of this process is a reorganization of oocyte microtubules such that microtubule density is highest at the anterior margin, microtubule minus-ends are enriched along the cortex, and microtubule plus-ends are enriched at the posterior pole (Steinhauer and Kalderon 2006).

As noted above, this pattern of microtubule organization was completely disrupted when Egl was depleted from stage 5 onwards. We therefore examined grk mRNA and protein localization in these egg chambers. In control egg chambers, grk mRNA was highly enriched at the posterior of the oocyte (Fig. 7A). grk mRNA was still enriched within oocytes of Egl depleted stage 5 egg chambers. However, the mRNA was not restricted to the posterior pole (Fig. 7B). Grk protein preferentially accumulated within the oocyte of control egg chambers (Fig. 7C, E). A similar qualitative localization pattern was observed for Grk protein in Egl depleted oocytes (Fig. 7D). However, quantification revealed that the oocyte enrichment of Grk was modestly reduced in Egl depleted egg chambers (Fig. 7E). Thus, depletion of Egl in stage 5 egg chambers results in subtle defects in the localization of grk mRNA and in the oocyte enrichment of Grk protein.

We next determined whether posterior cell fates could be specified in Egl depleted egg chambers. The pointed-LacZ (pnt-LacZ) enhancer trap strain was used for this analysis. LacZ signal from this strain is induced in posterior follicle cells in a Grk-dependent manner (Gonzalez-Reyes and St Johnston 1998; Becalska and Gavis 2010). Control egg chambers as well as Egl depleted egg chambers contained posterior LacZ signal (Figs. 7F, G, Supplemental Fig. 2H, I). Thus, despite a subtle defect in the oocyte enrichment of Grk protein, posterior follicle cell fate is specified in Egl depleted egg chambers.

Once specified, the posterior follicle cells signal back to the oocyte. This results in the recruitment of Par1 kinase to the posterior of stage 7 egg chambers (Doerflinger et al. 2006). Par1, along with additional members of the Par complex, function within the oocyte to reorganize oocyte microtubules (St Johnston and Ahringer 2010). We therefore examined the localization of Par1-GFP in control and Egl depleted stage 7 egg chambers. As expected, Par1-GFP was localized on the plasma membrane and enriched at the posterior of the oocyte in control egg chambers (Fig. 7H). By contrast, in Egl depleted oocytes Par1-GFP was neither localized on the plasma membrane nor enriched at the posterior pole (Fig. 7I).

Collectively, these results suggest that Egl is required for orchestrating the reorganization of microtubules that takes place in stage 7 egg chamber. At least one aspect of this function involves the posterior recruitment of Par1.

A specific role for Egl in mRNA localization in stage 10 egg chambers.

The maternal alpha-tubulin driver used in the preceding sections is located on the third chromosome. This driver is not expressed in the germarium, but is turned on in early stage egg chambers after oocyte specification (Supplemental Fig. 1A). For simplicity, we will refer to this strain as the “early-stage driver”. As shown previously, expression of egl shRNA-1 using this driver results in depletion of endogenous Egl from stage 5 onward (Fig. 2B).

An additional maternal alpha-tubulin driver is available from the Bloomington Drosophila stock center. This driver is present on the second chromosome, and is expressed at later stages of egg chamber maturation, from stages 5 or 6 onwards (Sanghavi et al. 2013).
For simplicity, we will refer to this strain as the “mid-stage driver”. The difference in expression profile between these drivers most likely results from position effects determined by their respective genomic locations. Nevertheless, expression of egl shRNA-1 using the mid-stage driver resulted in stage 10 egg chambers that were almost completely devoid of endogenous Egl within the germline (Fig. 8B, E). Quantification revealed that the level of depletion was similar to what was observed using the early-stage driver (Fig. 2E). By contrast, the level of endogenous Egl in stage 5 and 7 egg chambers was only modestly reduced using the mid-stage driver (Fig. 8A, C, D). Consistent reduction in the level of Egl was observed between stages 8 and 9 (data not shown).

We first examined microtubule organization in these Egl depleted egg chambers. As noted previously, the initiating event in reorganization of oocyte microtubules is the oocyte enrichment of grk mRNA and protein in stage 5 egg chambers. In contrast to what was observed using the early-stage driver, grk mRNA localization and Grk protein accumulation within the oocyte was unaffected upon expression of egl shRNA-1 using the mid-stage driver (Supplemental Fig. 5A-E). Consistent with this finding, the overall microtubule organization in stage 10 egg chambers resembled wild-type (Fig. 8F, G). Furthermore, microtubule plus-ends were enriched at the posterior pole and microtubule minus-ends were detected around the cortex in these Egl depleted egg chambers (Fig. 8H, I). In addition, the cortical localization of BicD was restored and Dhc was enriched at the posterior pole in egg chambers expressing egl shRNA-1 using the mid-stage driver (Fig. 8J, K). Thus, despite the fact that these stage 10 egg chambers are significantly depleted of Egl, their microtubule organization appears unaffected. Based on these results, we conclude that Egl is required between stages 5 through 7 for establishing the proper organization of oocyte microtubules. However, once this organization has been established, Egl does not appear to be required for maintaining microtubule organization in stage 10 egg chambers. One potential limitation of this conclusion, however, is that subtle defects might be present in the organization of oocyte microtubules that are not detected by our assays.

We next examined the localization of osk mRNA in stage 10 egg chambers expressing egl shRNA-1 using the mid-stage driver. In 58% of these egg chambers, osk mRNA was delocalized throughout the oocyte with residual enrichment at the posterior pole (Fig. 8L, M). Additionally, in 12% of egg chambers, osk mRNA was completely delocalized within the entire egg chamber with no residual posterior enrichment (Fig. 8N). Thus, Egl is required in stage 10 egg chambers for the correct posterior localization of osk mRNA. We next examined these egg chambers using the anti-Osk antibody. A reduced level of Osk protein was detected at the posterior pole of these Egl depleted egg chambers in comparison to the control (Fig. 8O, P). However, signal for Osk was not detected within the interior of the oocyte (Fig. 8P). Thus, the delocalized osk mRNA present in these oocytes is not translated. This result suggests that the role of Egl in regulating the translation of osk mRNA is either stage-specific, or that the translation of delocalized osk mRNA is indirectly caused by defects in microtubule organization. Although both scenarios are equally plausible, the latter scenario is consistent with the findings of Becalska et al (Becalska and Gavis 2010).

Becalska and Gavis recently demonstrated a link between the translational regulation of osk mRNA and microtubule organization (Becalska and Gavis 2010). In addition to osk mRNA, Egl is also required in stage 10 egg chambers for the correct localization of grk and bcd mRNAs. In 62% of stage 10 egg chambers expressing egl shRNA-1 using the mid-stage driver, grk mRNA was delocalized within the oocyte with
residual enrichment along the dorsal-anterior margin (Fig. 8Q, R). In a smaller percentage of egg chambers (27%), grk mRNA was completely delocalized throughout the entire egg chamber with no obvious dorsal-anterior localization (Fig. 8S). Consistent with the delocalization of grk mRNA, a significantly reduced level of Grk protein was detected at the dorsal-anterior corner of the oocyte (Fig. 8T, U). Similar finding were obtained for bcd mRNA; 75% of egg chambers displayed residual enrichment of bcd mRNA at the anterior cortex, whereas in 18% of egg chambers the mRNA was completely delocalized (Fig 8V-X).

Collectively, our results suggest that the efficient localization of osk, grk and bcd mRNAs in stage 10 egg chambers requires Egl.
Discussion
Previous work has shown that Egl performs an essential function in specification and maintenance of oocyte fate (Theurkauf et al. 1993; Carpenter 1994; Navarro et al. 2004). Egl performs these functions within the germarium and early stage (stages 1-3) egg chambers respectively. Using targeted depletion of Egl at various stages of egg chamber maturation, we have expanded on these studies. We demonstrate that Egl is required for the reorganization of oocyte microtubules that takes place in stage 7 egg chambers. By contrast, if Egl is present within early stage egg chambers, but depleted during stages 9 and 10, microtubule organization appears unaffected. However, localization of osk, bcd and grk mRNAs is disrupted. The localization of these mRNAs is critical for polarization of late stage oocytes and resulting embryos. Thus, formation of the mature Drosophila egg involves multiple functions of Egl at various stages of egg chamber maturation.

Egl and oocyte microtubules.
Egg chambers from loss-of-function egl mutants fail to specify an oocyte, and instead contain sixteen nurse cells (Theurkauf et al. 1993; Carpenter 1994). In fact, this phenotype is the source of the name ‘Egalitarian’ (all are equal, no special cell has been set aside as the oocyte). The precise mechanism by which Egl functions to establish oocyte fate is unknown. However, the available data suggests that this function likely involves regulation of the microtubule cytoskeleton (Theurkauf et al. 1993). Milder mutants in egl that are defective in binding Dynein light chain (Dlc) initially specify an oocyte (Navarro et al. 2004). However, oocyte fate is not maintained in these early egg chambers, and the oocyte ultimately reverts to a nurse cell fate (Navarro et al. 2004).

In this study, we demonstrate that Egl has additional roles in development of the Drosophila egg chamber. Depletion of Egl from stage 5 onwards has no effect on oocyte maintenance. The oocyte marker, Orb, is selectively localized within a single cell, the DNA within the oocyte nucleus retains its condensed morphology, and border cell migration occurs normally (Supplemental Fig. 2J-M, and data not shown). Border cells are a group of follicle cells that migrate from the anterior of the egg chamber towards the oocyte by a mechanism that involves sensing chemo-attractive cues produced by the oocyte (Montell 2006). Thus, if border cells migrate normally, it stands to reason that the oocyte is present and is functioning properly within this context. Despite this, several factors that are normally asymmetrically sorted become delocalized in the absence of Egl. These include mRNAs such as osk, bcd and grk (Fig. 3). In addition, the localization of motors and motor associated factors are also affected (Fig. 5). How might this occur? One possibility is that Egl is directly involved in the transport of each of these molecules. An alternative, and more plausible explanation, is that the delocalization of these factors is brought about by the severely disorganized microtubules present in Egl depleted oocytes.

Oocyte microtubules undergo a reorganization at stage 7 such that microtubule density becomes highest at the anterior of the oocyte, minus-ends become enriched around the cortex, and plus-ends become enriched at the posterior pole (Steinhauer and Kalderon 2006). This process appears to be completely disrupted if Egl is depleted from stage 5 egg chambers onward (Fig. 6). We envision two scenarios by which this could occur. One possibility is that Egl somehow directly functions to organize the arrangement and/or nucleation of oocyte microtubules. Consistent with this scenario is the finding that Egl is required within the germarium for directing the localization of the microtubule organizing center to the presumptive oocyte (Theurkauf et al. 1993). Also consistent with this notion is
our observation that microtubule density within the oocyte is significantly lower in stage 5 egg chambers that have been depleted of Egl (Supplemental Fig. 2N, O).

Another possibility is that Egl is required for the enrichment of grk mRNA and protein within the oocyte and a defect in these processes indirectly affects microtubules. Indeed, grk mRNA is not correctly localized in Egl depleted stage 5 egg chambers. Although grk mRNA in enriched within these oocytes, unlike the control, it is not localized at the oocyte posterior (Fig. 7A, B). In addition, the oocyte enrichment of Grk protein is modestly reduced in Egl depleted stage 5 egg chambers (Fig. 7C-E). However, these deficits do not appear to affect the Grk-dependent induction of posterior follicle cell fate (Fig. 7F, G). Thus, it is unlikely that Egl contributes to microtubule organization by affecting this arm of the pathway. Once posterior follicle cell fate has been established, these cells signal back to the oocyte, resulting in the recruitment of Par1 kinase to the posterior of the oocyte (Doerflinger et al. 2006). This process is severely affected in Egl depleted egg chambers (Fig. 7H, I). Thus, Egl functions within the oocyte to facilitate the reorganization of microtubules in response to the posterior follicle cells signal.

It is worth noting that depletion of Egl produces a unique phenotype with respect to microtubule organization. As stated previously, grk mutants fail to reorganize oocyte microtubules at stage 7. However, in grk mutants, osk mRNA is delocalized to a central focus within the oocyte and bcd mRNA is present at both poles (Gonzalez-Reyes et al. 1995; Roth et al. 1995). By contrast, osk mRNA is uniformly distributed throughout the entire egg chamber in Egl depleted oocytes (Fig. 3B). In addition, bcd mRNA is either uniformly distributed or enriched along the oocyte cortex in Egl depleted egg chambers (Fig. 3J, K). The common phenotype between Egl depletion and grk mutants is the defective migration of the oocyte nucleus away from the posterior pole (Fig. 5G) (Gonzalez-Reyes et al. 1995; Roth et al. 1995).

Furthermore, in par1 and baz/par3 mutants, osk mRNA is delocalized to a central focus (Shulman et al. 2000; Tomancak et al. 2000; Becalska and Gavis 2010; Doerflinger et al. 2010). In some mutants alleles of these genes, bcd mRNA is relatively unaffected (Shulman et al. 2000), whereas in others, bcd mRNA localizes along the lateral cortex in a similar manner to Egl depletion (Becalska and Gavis 2010; Doerflinger et al. 2010). In some mutant alleles of baz, the oocyte nucleus is retained at the posterior (Becalska and Gavis 2010; Doerflinger et al. 2010), whereas in some mutants of par-1, migration of the oocyte nucleus is unaffected (Shulman et al. 2000). These varying results highlight the need for caution in interpreting the role of these genes in microtubule reorganization. The strength and nature of the phenotype will likely depend on the strength and nature of the mutant allele that was used. In addition, with an shRNA-based approach, the phenotypes will depend of the efficacy and timing of knock-down. Thus, we cannot conclude with certainly that the phenotype we describe in this report arises from complete depletion of Egl from stages 5 onward. A residual level of Egl might persist in these egg chambers and produce a milder phenotype than what would be observed if Egl could be completely eliminated.

An important future question will be to determine the molecular mechanism by which Egl functions in microtubule organization.

Egl and mRNA localization.

As noted previously, Egl has been implicated in the Dynein-dependent localization of mRNAs in the Drosophila embryo (Dienstbier et al. 2009). We therefore investigated
whether Egl might perform a similar function in the oocyte. Consistent with a role for Egl in mRNA localization, Egl-GFP was found to preferentially associate with localized mRNAs (Fig. 1D). In addition, depletion of Egl from stage 5 onwards resulted in complete delocalization of osk, bcd and grk mRNAs (Fig. 3). However, as noted in the previous section, microtubules were severely disorganized in this background. Thus, from this experiment, we cannot conclude whether Egl is specifically required for mRNA localization. By contrast, depletion of Egl from stage 8 onwards does not appear to affect the organization of oocyte microtubules (Fig. 8F-I). Despite this, osk, bcd, and grk mRNAs were all significantly delocalized in these egg chambers (Fig. 8). Our results therefore suggest that, much like in the embryo, Egl functions in mRNA localization within the oocyte. Because the efficient localization of all three mRNAs requires the Dynein motor (Duncan and Warrior 2002; Januschke et al. 2002; MacDougall et al. 2003; Weil et al. 2006; Sanghavi et al. 2013), it is likely that Egl performs this function via its association with Dynein (Navarro et al. 2004).

**Egl and translation regulation.**

An unexpected finding was that Egl was required for the translational regulation of osk and grk mRNAs. Depletion of Egl from stage 5 onwards resulted in translation of delocalized osk mRNA (Fig. 4C). By contrast, delocalized grk mRNA in these same oocytes appeared to be inefficiently translated (Fig. 4J). How might Egl function in regulating the translation of these mRNAs? One possibility is a direct role for Egl in this process. A potential role for Egl in translation regulation was postulated by Huynh and St Johnston (Huynh and St Johnston 2000). It is also possible that Egl participates indirectly in this process. As noted previously, depletion of Egl from stage 5 onwards resulted in profound defects in microtubule organization (Fig. 6). Interestingly, Beckalska et al., have demonstrated a correlation between microtubule organization and translation of osk mRNA (Becalska and Gavis 2010). In general, mutants that affect microtubule organization, cause ectopic translation of osk mRNA (Becalska and Gavis 2010). Similarly, mutants with defective microtubule organization have been shown to affect the translation of grk mRNA. In these mutants, grk mRNA is inefficiently translated (Abdu et al. 2006; Klattenhoff et al. 2007; Pane et al. 2007). It is therefore possible that the aberrant microtubule organization present in these Egl depleted egg chambers underlies the defective translational regulation of osk and grk mRNAs. Consistent with this notion, depletion of Egl from stage 8 onwards using the mid-stage driver resulted in significant delocalization of osk mRNA. However, microtubules were correctly organized in these egg chambers, and the delocalized osk mRNA remained translationally repressed (Fig. 8M-P).

**A model for Egl function in the Drosophila female germline.**

Published results have indicated a role for Egl within the germarium in establishment of oocyte fate (Fig. 9) (Theurkauf et al. 1993; Carpenter 1994; Mach and Lehmann 1997; Huynh and St Johnston 2000; Bolivar et al. 2001). In early stage egg chambers (stages 1-3), Egl is required for maintaining oocyte fate (Fig. 9) (Navarro et al. 2004). During mid-stages of oogenesis (stages 5-7), we demonstrate that Egl is required within the oocyte to facilitate the reorganization of microtubules in response to the signal from posterior follicle cells (Fig. 9). Finally, during stages 9 and 10, Egl is required for the efficient localization of osk, bcd, and grk mRNAs (Fig. 9). The precise localization of these mRNAs is critical for establishing and maintaining the polarity of the oocyte and the future embryo. Thus, Egl performs essential functions at multiple stages during development of the Drosophila egg.
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Figure Legends

Fig. 1 The localization and mRNA binding properties of Egl.

(A) A schematic of a Drosophila ovariole. The germline stem cells and their niche reside at the anterior tip of the ovariole in a region known as the germarium. The stem cell divides to produce a daughter cell known as a cystoblast. The cystoblast undergoes 4 rounds of cell division with incomplete cytokinesis to produce a cyst containing 16 germ cells. One of these 16 germ cells will become the oocyte (red cell); the rest will assume nurse cell fate. During maturation within the germarium, the oocyte comes to reside at the posterior of the cyst. Also within the germarium, the cyst becomes surrounded by a layer of somatic cells known as follicle cells. This structure is now referred to as an egg chamber. The egg chamber progresses through 14 stages of morphogenesis before it is competent for fertilization. Egg chambers from the following stages are indicated in the schematic: Stage 2 (S2), Stage 4 (S4), Stage 5 (S5), Stage 7 (S7), Stage 9 (S9), and Stage 10A (S10A). Between stages 5 and 7, signaling events between the oocyte and the overlying follicle cells result in reorganization of oocyte microtubules. As a consequence, the oocyte nucleus migrates from the posterior to the dorsal-anterior margin (blue circle).

(B) A representative Drosophila ovariole from a wild-type strain. The egg chambers were stained with an antibody against gamma-tubulin.

(C) Ovaries were dissected from wild-type flies, fixed and processed for immunofluorescence using antibodies against Dynein heavy chain (Dhc, green) and Egl (red). The arrow indicates enrichment of Dhc at the posterior pole.

(D) Ovaries were dissected from flies expressing either GFP or Egl-GFP in the female germline. Lysates were prepared and incubated with GFP-trap beads. The co-precipitating RNAs were reverse transcribed and processed for quantitative PCR using primers against the indicated genes. The level of co-precipitating rp49, osk, bcd and grk were normalized to the level of co-precipitating gamma-tubulin mRNA. rp49 and gamma-tubulin represent mRNAs that are not asymmetrically localized within the egg chamber. The entire experiment was done in triplicate. The error bars represent standard deviation. * p = 0.033, ** p = 0.0013, and *** p = 0.0009, unpaired t test. The scale bar in these figures represents 50 microns.

Fig. 2 Germline specific depletion of Egl.

(A-D) Egg chambers expressing either a control shRNA (A, C) or egl shRNA-1 (B, D) were fixed and processed for immunofluorescence using an antibody against Egl. The control used in this and in following experiments corresponds to a strain expressing shRNA against eb1. Expression of shRNAs was under control of a maternal alpha-tubulin driver that is active from early stage egg chambers onwards (see Supplemental Fig. 1A). The left inset in panel B represents a magnified view of the area indicated by the white lines. The right inset in panel B shows a stage 5/6 egg chamber from a different ovariole from flies expressing egl shRNA-1. The arrow in panel D indicates the overlying main body follicle cells. The arrowhead in panel D
indicates the border cells. Also indicated in D is the % of stage 10 egg chamber displaying convincing depletion of Egl within the germline.

(E) The level of Egl within the germline was quantified using the following strains: a strain expressing a control shRNA, a strain expressing egl shRNA-1 using the early-stage driver, and a strain expressing egl shRNA-1 using the mid-stage driver (see Supplemental Fig. 1B). Ovaries were fixed and processed for immunofluorescence using an antibody against Egl. The fluorescence intensity of Egl immuno-staining was measured within the germline of 25 different egg chambers from each strain. Error bars represent standard deviation. *** p<0.0001, unpaired t test. The scale bar in these figures represents 50 microns.

Figure 3. mRNA localization defects in Egl depleted egg chambers.
(A-B) Egg chambers expressing either a control shRNA (A) or egl shRNA-1 (B) using the early-stage driver were fixed and processed for in situ hybridization using an anti-sense probe against osk mRNA (green). The egg chambers were counterstained with ToPro3 to reveal nuclei (red). The arrow in panel A indicates the normal localization of osk mRNA at the posterior pole.

(C) In order to examine the specificity of the in situ hybridization signal, ovaries from egl shRNA-1 expressing flies were fixed and processed for in situ hybridization using a sense probe against osk mRNA (green). The egg chambers were also counterstained with ToPro3 (red).

(D-F) Egg chambers expressing a control shRNA (D), egl shRNA-1 (E), or egl shRNA-2 (F) were fixed and processed for immunofluorescence using an antibody against Staufen (Stau, green). The egg chambers were also counterstained with phalloidin to reveal F-actin (red). The arrows in panels D and F indicate the posterior localization of Stau in control and egl shRNA-2 expressing egg chambers. Also indicated in panel E is the penetrance of the phenotype and the number of egg chambers that were scored.

(G-H) Egg chambers expressing a control shRNA (G) or egl shRNA-1 (H) using the early-stage driver were fixed and processed for in situ hybridization using an anti-sense probe against grk mRNA (green). The samples were counterstained with ToPro3 (red). The arrow in panel G indicates the normal localization of grk mRNA at the dorsal-anterior corner of the oocyte.

(I-K) The same two strains used in the above panels were also examined using an anti-sense probe against bcd mRNA (green). ToPro3 was used to visualize nuclei (red). The arrow in panel I indicates the normal localization of bcd mRNA at the anterior margin of the oocyte. In 42% of egg chambers expressing egl shRNA-1, bcd mRNA was delocalized throughout the entire egg chamber (J). In 53% of egg chambers expressing egl shRNA-1, bcd mRNA accumulated along the cortex (K, arrows)

(L) The graph represents quantification of mRNA localization phenotypes. The two strains analyzed were ones expressing either a control shRNA or egl shRNA-1 using
the early-stage driver. The green bars represent a wild-type localization pattern (examples in panel A, G, and I). The red bars represent egg chambers containing mRNA that was diffusely distributed throughout the entire egg chamber (examples are panel B, H, and J). The yellow bar represents mRNA that was detected along the cortex (panel K). The number of egg chambers scored for each genotype is indicated. The scale bar in these figures represents 50 microns.

Figure 4. The role of Egl in translation regulation.
(A-D) Egg chambers from the following strains were fixed and processed for immunofluorescence using a chicken anti-Osk antibody: a strain expressing a control shRNA (A), osk<sup>84</sup>/osk Def (B, an osk protein-null strain), a strain expressing egl shRNA-1 using the early-stage driver (C) and a strain expressing egl shRNA-2 using the early-stage driver (D). Arrows in A and D indicate the normal posterior localization of Osk protein.

(E-H) Ovaries from flies expressing a control shRNA (E, G) or egl shRNA-1 (F, H) were fixed and processed for immunofluorescence using a mouse anti-Osk antibody (E, F) or a rabbit anti-Osk antibody (G, H).

(I-K) Ovaries from flies expressing a control shRNA (I), egl shRNA-1 (J), or khc shRNA (K) were fixed and processed for immunofluorescence using an antibody against Grk (grey-scale image). The egg chambers were also counterstained with DAPI to visualize nuclei (red). The arrow in panel I indicates the normal localization of Grk protein at the dorsal-anterior corner of the oocyte. The arrow in panel K represents the delocalized Grk protein in Khc depleted oocytes.

(L). Ovaries from flies expressing khc shRNA were fixed and processed for immunofluorescence using a chicken anti-Osk antibody. The number of egg chambers scored as well as the penetrance of each phenotype is indicated. The scale bar in these figures represents 50 microns.

Figure 5. Delocalization of Dynein, Kinesin, BicD, and the oocyte nucleus in Egl depleted egg chambers.
(A-B) Ovaries from flies expressing a control shRNA (A) or egl shRNA-1 (B) were fixed and processed for immunofluorescence using antibodies against Dhc (red) and Khc (A', B', green). A merged image is also shown (A'', B'').

(C-D) Ovaries from flies expressing a control shRNA (C) or egl shRNA-1 (D) were fixed and processed for immunofluorescence using an antibody against BicD.

(E-G) Ovaries from flies expressing a control shRNA (E) or egl shRNA-1 (F, G) were fixed and processed for immunofluorescence using an antibody against Lamin DmO (green). The egg chambers were also counterstained with Phalloidin (red). The arrow in panel E indicates the normal localization of the oocyte nucleus. The arrows in panels F and G indicate the mis-localized oocyte nucleus in Egl depleted egg chambers. The number of egg chambers scored as well as the penetrance of each phenotype is indicated. The scale bar in these figures represents 50 microns.

Figure 6. Microtubule organization in Egl depleted egg chambers.
(A-C) Ovaries from flies expressing a control shRNA (A) or egl shRNA-1 (B, C) were fixed and processed for immunofluorescence using an antibody against alpha-tubulin. There is a high density of microtubules at the anterior margin of control oocytes (A, arrow). The posterior of control oocytes contain a much lower density of microtubules (A, asterisk).

(D-E') Ovaries from flies expressing a control shRNA (D) or egl shRNA-1 (E, E') were fixed and processed for immunofluorescence using an antibody against Eb1. The arrow in panel D indicates the posterior enrichment of Eb1 foci in control egg chambers.

(F-G) Ovaries from control flies expressing Kinesin beta-gal (F, Kin:βgal, red) or Kinesin beta-gal along with egl shRNA-1 (G) were fixed and processed for immunofluorescence using an antibody against beta galactosidase. The egg chambers were also counterstained with DAPI (green). The arrow in panel F indicates the posterior localization of Kin:βgal in control egg chambers.

(H-M) Ovaries from flies expressing a control shRNA (H, J, L) or egl shRNA-1 (I, K, M) were fixed and processed for immunofluorescence using an antibody against gamma-tubulin. The arrows in panel H indicate the cortical enrichment of gamma-tubulin in a control stage 10 egg chambers. The asterisk in panel I indicates the mis-localized oocyte nucleus. The white arrow in panels J and K indicate a stage 5 egg chamber and the red arrows in these panels indicate a stage 7 egg chamber. The arrows in panel L indicate the cortical enrichment of gamma-tubulin in an early stage 9 egg chamber.

(N-P) Ovaries from flies expressing GFP-Cnn (N) or from flies expressing GFP-Cnn and egl shRNA-1 (O, P) were fixed and processed for immunofluorescence using an antibody against GFP. Expression of the shRNA was under control of the early-stage maternal alpha tubulin driver. Arrows in panel N indicate the cortical enrichment of GFP-Cnn in the control strain. The arrow in panel P indicates the residual cortical enrichment of GFP-Cnn in a subset of Egl depleted egg chamber. The asterisk in this panel indicates the mis-localized oocyte nucleus. The number of egg chambers scored as well as the penetrance of each phenotype is indicated. The scale bar in these figures represents 50 microns.

Fig. 7. The role of Egl in microtubule organization.

(A-B) Ovaries from flies expressing a control shRNA (A) or egl shRNA-1 (B) using the early-stage driver were fixed and processed for in situ hybridization using an anti-sense probe against grk mRNA (green). The egg chambers were counterstained with ToPro3 (red).

(C-D) Ovaries from flies expressing a control shRNA (A) or egl shRNA-1 (B) using the early-stage driver were fixed and processed for immunofluorescence using an antibody against Grk (green). The egg chambers were counterstained with DAPI (red).
(E) The enrichment of Grk within the oocyte of control egg chambers was compared to that from Egl depleted egg chambers. For each genotype, 15 egg chambers were quantified. ** p = 0.0022, unpaired t test.

(F-G) Ovaries from flies expressing pnt-LacZ (F) or from flies expressing pnt-LacZ and egl shRNA-1 using the early-stage driver (G) were fixed and processed for immunofluorescence using an antibody against beta-galactosidase (cyan). The ovaries were also counterstained with DAPI (red). F’ and G’ represent a magnified view of the posterior follicle cells in panels F and G respectively.

(H-I) Ovaries from flies expressing Par1-GFP (H) or from flies expressing Par1-GFP and egl shRNA-1 using the early-stage driver (I) were fixed and processed for immunofluorescence using an antibody against beta-galactosidase (grey). The ovaries were also counterstained with DAPI (red). In panel H’ and I’, the DAPI and GFP signals are overlaid. The arrowhead in panel H indicates the membrane localization of Par-GFP in the control strain. The arrow in panel H indicates the posterior enrichment of Par1-GFP in control egg chambers. The penetrance of each phenotype and the number of egg chambers scored is indicated. The scale bar in panels F and G represent 50 microns. In the rest of the panels, the scale bar represents 25 microns.

Figure 8. Egl is required in stage 10 egg chambers for mRNA localization.

(A-E) Ovaries from flies expressing a control shRNA (A, B) or egl shRNA-1 (C-E) were fixed and processed for immunofluorescence using an antibody against Egl. Panels A and C represent stage 5 egg chambers. Panel D represents a stage 7 egg chamber and panels B and E represent stage 10 egg chambers. Expression of the shRNAs was driven using the mid-stage driver (see Supplemental Fig. 1B).

(F-G) Ovaries from flies expressing a control shRNA (F) or egl shRNA-1 (G) using the mid-stage driver were fixed and processed for immunofluorescence using an antibody against alpha-tubulin (green).

(H) Egg chambers from flies co-expressing Kin;βgal and egl shRNA-1 using the mid-stage driver were fixed and processed for immunofluorescence using an antibody against beta galactosidase (green). The egg chambers were also counterstained with DAPI (red).

(I-K) Ovaries from flies expressing egl shRNA-1 (I, J, K) using the mid-stage driver were fixed and processed for immunofluorescence using antibodies against gamma-tubulin (I), Dhc (J), and BicD (K).

(L-N) Ovaries from flies expressing a control shRNA (L) or egl shRNA-1 (M, N) using the mid-stage driver were fixed and processed for in situ hybridization using an antisense probe against osk mRNA (green). The egg chambers were counterstained with ToPro3 (red). The arrow in panel L indicates the posterior localization of osk mRNA in control strains. The arrow in panel M indicates the residual posterior enrichment of osk mRNA in a subset of Egl depleted egg chambers.
(O-P) The same strains used in the preceding panels were fixed and processed for immunofluorescence using an antibody against Osk. The arrow in panel P indicates the residual posterior Osk observed in Egl depleted egg chambers.

(Q-S) Ovaries from flies expressing a control shRNA (Q) or egl shRNA-1 (R, S) using the mid-stage driver were fixed and processed for in situ hybridization using an anti-sense probe again grk mRNA (green). The egg chambers were counterstained with ToPro3 (red). The arrow in Q indicates the dorsal-anterior localization of grk mRNA in control strains. The arrow in R indicates the residual enrichment of grk at the dorsal-anterior corner in a subset of Egl depleted egg chambers.

(T-U) The same strains used in the preceding panels were fixed and processed for immunofluorescence using an antibody against Grk. The arrow in U indicates the residual Grk protein at the dorsal-anterior corner of Egl depleted egg chambers.

(V-X) Ovaries from flies expressing a control shRNA (V) or egl shRNA-1 (W, X) using the mid-stage driver were fixed and processed for in situ hybridization using an anti-sense probe again bcd mRNA (green). The egg chambers were also counterstained with ToPro3 (red). The arrow in V indicates the anterior localization of bcd mRNA in control strains. The arrow in W indicates the residual anterior enrichment of bcd in a subset of Egl depleted egg chambers. The number of egg chambers scored and the penetrance of the phenotypes are indicated. The scale bar in these figures represents 50 microns.

**Figure 9. Model for Egl function in maturation of the Drosophila egg chamber.** Based on the findings in this report as well as published results, we conclude that Egl has at least four separate functions during egg chamber maturation.

1. Within the germarium, Egl is required for oocyte specification (Theurkauf et al. 1993; Carpenter 1994).
2. In early stage egg chambers, Egl is also required for maintaining oocyte fate (Navarro et al. 2004).
3. Between stages 5 and 7, Egl is required for reorganization of oocyte microtubules.
4. In stage 9 and 10 egg chambers, Egl is required for the correct spatial localization of osk, bcd and grk mRNAs.

**Supplemental Fig. 1. Expression profile of drivers used.**

(A) Ovaries from flies expressing Act5c-mRFP (red) using the early-stage maternal alpha-tubulin driver (Bloomington stock #7063) were fixed and stained with DAPI to reveal nuclei (blue).

(B). Flies expressing Act5c-mRFP (red) using the mid-stage maternal alpha-tubulin driver (Bloomington stock #7062) were fixed and stained with DAPI to reveal nuclei (blue). In both panels, the red signal indicates fluorescence from mRFP.

**Supplemental Fig. 2. Egl depletion phenotypes.**

(A) Ovaries from flies expressing a control shRNA (lane 1) or egl shRNA-1 (lane 2) using the early-stage driver were dissected and lysates were prepared. The lysates were analyzed by western blotting using the indicated antibodies.
(B) Ovaries from flies expressing egl shRNA-2 using the early-stage driver were fixed and processed for immunofluorescence using an antibody against Egl.

(C) Total RNA was extracted from flies expressing a control shRNA (blue bars) or from flies expressing egl shRNA-1 using the early-stage driver (red bars). The RNA was reverse transcribed using random hexamers. The cDNA was analyzed by quantitative PCR using primes against osk, bcd and grk. The level of osk, bcd and grk between the two strains was normalized to the level of gamma-tubulin and rp49. There is no statistically significant difference in the level of osk, bcd and grk between these strains.

(D-G) Ovaries from flies expressing a control shRNA (D, F) or egl shRNA-1 (E, G) using the early-stage driver were fixed and processed for immunofluorescence using either a chicken anti-Osk antibody (D, E) or a mouse anti-Osk antibody (F, G).

(H-I) Ovaries from flies expressing pnt-LacZ (H) or from flies expressing pnt-LacZ and egl shRNA-1 using the early-stage driver (I) were fixed and processed for immunofluorescence using an antibody against beta-galactosidase (red). The ovaries were also counterstained with DAPI (cyan, H’ and I’).

(J-M) Ovaries from flies expressing a control shRNA (J, K) or from flies expressing egl shRNA-1 using the early-stage driver (L, M) were fixed and processed for immunofluorescence using an antibody against Orb (red). The egg chambers were also counterstained with DAPI (green).

(N-O) The same two strains used in the above panels were fixed and stained with an antibody against alpha-tubulin to reveal the microtubule network. The scale bar in these images represents 50 microns.

Supplemental Fig. 3. Rescue of Egl depletion phenotypes.
(A) Schematic indicating the recognition site for egl shRNA-1. In order to create an egl transgenic construct that was refractory to the shRNA (eglR-GFP), the residues indicated in blue were mutated. These residues are at the wobble position of the coding sequence and the resulting nucleotide changes do not affect the amino acid sequence of Egl.

(B) Ovaries were dissected from a strain that co-expressed egl shRNA-1 and the eglR-GFP construct. The shRNA as well as EglR-GFP were expressed using the early-stage driver. For simplicity, we will refer to this as the “rescue strain”. This panel indicates the localization of EglR-GFP.

(C) Ovaries from the rescue strain were fixed and processed for in situ hybridization using an anti-sense probe against osk mRNA (red). The samples were also counterstained with ToPro3 (green).
(D) Ovaries from the rescue strain were fixed and processed for immunofluorescence using antibodies against Stau (red).

(E-F) Ovaries from the rescue strain were fixed and processed for in situ hybridization using anti-sense probes against grk mRNA (E, red) and bcd mRNA (F, red). The samples were also counterstained with ToPro3 (green).

(G) Ovaries from the rescue strain were fixed and processed for immunofluorescence using a chicken anti-Osk antibody (red).

(H-I) Ovaries from the rescue strain were fixed and processed for immunofluorescence using antibodies against Dhc (H) and BicD (I).

(J) Ovaries from flies co-expressing Kin::βgal, egl shRNA-1, and eglR-GFP were fixed and processed for immunofluorescence using an antibody against beta galactosidase (red). The egg chambers were also counterstained with DAPI (green).

(K-L) Ovaries from the rescue strain were fixed and processed for immunofluorescence using antibodies against Eb1 (K) and gamma-tubulin (L). The scale bar on these images represents 50 microns.

**Supplemental Fig. 4. osk and grk mRNA localization in Khc depleted oocytes.**

(A-B) Egg chambers expressing a control shRNA (A) or khc shRNA (B) using the early-stage driver were fixed and processed for in situ hybridization using an anti-sense probe against osk mRNA (green). The samples were also counterstained with ToPro3 (red).

(C-D) The same strains used in the preceding panels were fixed and processed for in situ hybridization using an anti-sense probe against grk mRNA (green). The samples were counterstained with ToPro3 (red). The scale bar in these images is 50 microns.

**Supplemental Fig. 5. grk mRNA and protein localization in Egl depleted oocytes.**

(A-B) Egg chambers expressing a control shRNA (A) or egl shRNA-1 (B) using the mid-stage driver were fixed and processed for in situ hybridization using an anti-sense probe against grk mRNA (green). The dashed line indicates the outline of the egg chamber and the solid line indicates the outline of the oocyte.

(C-D) The same strains used in the above panels were fixed and processed for immunofluorescence using an antibody against Grk (green). The samples were also counterstained with DAPI (red). The scale bar on these images represents 25 microns.

(E) The Grk signal for egg chambers depicted in panels C and D was quantified. The enrichment of Grk within the oocyte was quantified. For each genotype, 15 egg
chambers were quantified. There is no statistical difference between these two strains.
References


Figure 1

A. Germarium

B. Gamma tubulin

C. Dhc, Egl, Merge

D. Graph showing fold change for rp49, oskar, gurken, bicoid with GFP and Egl GFP.
Figure 2
Figure 3
Figure 4
**Figure 5**

A. Control

A'. egl-shRNA-1

A''. Merge

B. egl-shRNA-1

B'. Dhc

B''. Khc

B'''. Merge

C. Control

D. egl-shRNA-1

D'. Bic-D

D''. Bic-D

E. Control

F. egl-shRNA-1

F'. Lamin/Actin

F''. Lamin/Actin

G. egl-shRNA-1

G'. Lamin/Actin

G''. Lamin/Actin

Data:

- A. 19% n=103
- B. 29% n=103
- C. 93% n=59
- D. 94% n=70
- E. 94% n=70
- F. 93% n=59
- G. 93% n=59
Figure 6
Figure 7
Figure 8
1. Specification of oocyte fate

If Egl is absent, cysts with 16 nurse cells are formed.

2. Maintenance of oocyte fate

Egl mutants that are defective in binding Dynein light chain.

Oocyte is initially specified. Reverts to nurse cell fate.

Most cysts degenerate.

3. Reorganization of oocyte microtubules

Depletion of Egl from stage 5 egg chambers onwards.

Oocyte fate is specified and maintained.

Microtubules do not reorganize in stage 7 egg chambers.

osk, bcd, grk mRNA and motors are delocalized.

Delocalized osk mRNA is translated into protein.

4. Localization of osk, bcd, and grk mRNAs

Depletion of Egl from stage 8/9 egg chambers onwards.

Oocyte fate is specified and maintained.

Microtubule organization is normal.

Motors are localized.

osk, bcd and grk mRNAs are delocalized.

Translational regulation of osk mRNA is maintained.

**Figure 9**
A. Endogenous *egl*  

\[1588\text{ACC-ACG-GTG-ATA-GCG-AAT-GTC}_{1608}\]  

Transgenic *eglR-WT*  

\[1588\text{ACC-ACG-GTG-ATC-GCG-AAT-GTC}_{1608}\]  

shRNA binding site  

site-directed mutagenesis  

Refractory wobble mutations

Supplemental Fig. 3
Supplemental Fig. 4
Fold enrichment of Grk in the oocyte

E. Supplemental Fig. 5