Investigating the role of transcription factor, *Trl*, during germline development in the *Drosophila* ovary

by

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Oogenesis is the process by which an egg develops from undifferentiated cells in the ovary. The *Drosophila melanogaster* ovary is an assembly line for egg production, making it a great system to investigate initial oocyte specification and oocyte growth and maturity. *Trl*, *GAGA factor*, encodes a transcription factor that blocks repressive histones by modifying chromatin accessibility. *Trl* is expressed throughout the fly and during all stages of development. *Trl* is necessary for eye and wing development, embryogenesis, and male germ cell development in spermatogenesis. Other studies have begun to characterize *Trl* in oogenesis; however, the specific role *Trl* plays in the germline has yet to be determined. In global mutants, *Trl* functions in oogenesis for follicle survival, proper germ cell number, nurse cell size, oocyte fate, and oocyte localization. Since *Trl* is necessary for early events in fly development, homozygous null mutants are lethal, I utilized the *Flippase/Flippase Recognition Target* (*Flp/FRT*)-mediated clonal analysis and tissue-specific RNAi to investigate how *Trl* germline-specific mutants regulate oocyte fate in the adult fly ovary. In this study, I found that *Trl* is not necessary for

germline stem cell maintenance. Loss of *Trl* in stages 2-4 and stages 7-9 results in condensed, pyknotic, fragmented nuclei in cysts, an indicator of premature cyst death. $Trl^{13C(40)}$ mutant cysts have a delay in Orb expression, whereas $Trl^{s2325}$ clones lack Orb altogether, indicating that *Trl* is necessary for Orb in the oocyte. However, *Trl* is not necessary for entry into meiosis or oocyte localization. Further, knock-down of *Trl* in stage 2 cysts using a germline-specific RNAi driven by *OtuGal4* did not block Orb expression, suggesting that *Trl* is not required for oocyte maintenance. Taken together, I conclude that *Trl* is necessary for Orb accumulation in the oocyte before 16-cell cyst formation. Understanding the role *Trl* plays in the oocyte can help us understand how chromatin modification affects the critical set of conserved, developmental steps a mature oocyte must undergo. In turn, it could also help us understand these developmental steps in other species.

# Investigating the role of transcription factor, *Trl*, during germline development in the *Drosophila* ovary

A Thesis

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East Carolina University

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Master of Science in Biology

by

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## TABLE OF CONTENTS

**LIST OF FIGURES**

## LIST OF SYMBOLS OR ABBREVIATIONS

| | |
|---|---|
| GSC | Germline stem cell |
| PGC | Primordial germ cell |
| CB | Cystoblast |
| NC | Nurse cell |
| OO | Oocyte |
| TF | Terminal filament |
| FSC | Follicle stem cell |
| SC | Synaptonemal Complex |
| FLP/FRT | Flippase/Flippase recognition target |
| #d AHS | days after heat shock/days after clone induction |
| RNAi | RNA interference |
| dae | days after eclosion |
| PCD | programmed cell death |
| PcG | Polycomb group genes |
| Trx | Tri-thorax group genes |
| Trl | Trithroax-like |
| GFP | Green Fluorescence Protein |
| BicD | Bicaudal D |
| Orb | oo18 RNA-binding protein |
| Egl | Egalitarian |
| Grk | Gurken |

| | |
|---|---|
| Baz | Bazooka (PAR-3) |
| C(3)G | crossover suppressor on 3 of Gowen |
| DAPI | 4′,6-diamidino-2-phenylindole |
| 1B1 | hu li tai shao (Hts) |
| LamC | Lamin C |
| PAR | Protease-activated receptor |
| H3K4me3 | histone 3 lysine 4 trimethylation |
| H3K27me3 | histone 3 lysine 27 trimethylation |
| ISWI | Imitation Switch |

## Introduction

Germ cells differ from somatic cells in that they can differentiate into gametes and eventually, when fertilized, an organism (Gleason, Anand, Kai, and Chen, 2018). The maturation of gametes is therefore essential for species survival and reproduction. Oogenesis is the process by which differentiation occurs to produce a fertilizable oocyte within the ovary (Belles and Piulachs, 2014). Oocytes undergo a critical set of developmental steps, and these steps are largely conserved across species (Pepling et al., 1999).

*Drosophila* is an excellent model for oocyte and germ cell development because *Drosophila* oogenesis is a well-characterized, tightly controlled process. The underlying mechanisms and genes for oocyte and germ cell development in *Drosophila* are conserved in humans as well as in other organisms (Jennings, 2011). *Drosophila* oogenesis involves similar developmental steps from primary follicle cell to a mature egg chamber as that of mammalian oogenesis (Grive and Freiman, 2015). *Drosophila* has a shorter generation time than mammalian models. There are also freely available genetic resources and reagents for ease of experimental design making *Drosophila* a great model with which to study oocyte and germ cell development in oogenesis.

Production of a mature oocyte in *Drosophila* is dependent upon the ability of germline stem cells (GSCs) to self-renew and proliferate. *Drosophila* ovaries are made of 14-16 individual ovarioles, which are an assembly line of increasingly developed oocytes (Figure 1A) (Spradling, 1993, Hudson and Cooley, 2014). Oocyte development starts at the anterior tip of the ovariole, called the germarium, where the GSCs reside (Figure 1B). GSCs are anchored via adherens junctions to somatic cells known as cap cells that are directly adjacent to the GSCs (Figure 1B) (Song et al., 2002). GSCs asymmetrically

divide to give rise to a daughter cell termed a cystoblast (CB) and an undifferentiated cell (Spradling, 1993, Gonzalez-Reyes, 2003, Xie, 2013). The cystoblast differentiates and undergoes four incomplete mitotic divisions, creating 2-, 4-, 8-, and 16-cell cysts (Spradling, 1993; Xie, 2013; Yamashita, 2018). The fusome, a cytoplasmic organelle, branches through each cell in the cyst with the help of ring canals or connections between neighboring cells within the cyst. The fusome maintains mitotic division synchronicity during incomplete divisions (Huynh, 2006). As mitotic divisions progress, somatic cells assist in moving the maturing cyst posteriorly through the germarium. The last mitotic cyst division results in a 16-cell cyst which becomes surrounded by a single layer of follicle cells, creating an egg chamber (Spradling, 1993) (Figure 1A-B). In the 16-cell cyst, one cell differentiates into an oocyte and becomes arrested in meiosis until completion of stage 10 of oogenesis. The remaining 15 cells become supporting nurse cells, which undergo endoreplication to create a mass of RNA and proteins that will be deposited in the oocyte before oogenesis is complete (Spradling, 1993). Egg chambers that leave the germarium continue to develop as they move to the posterior end of the ovariole. Egg chambers can be classified by their DNA morphology and overall size as 14 different stages of oogenesis, the first stage beginning at the posterior end of the germarium (region 3 of the germarium) (Figure 1A). The linear arrangement of egg chambers allows for visualization of defects in germ cell development.

Oocyte fate requires proper timing of entry into meiosis. Chromosome homolog pairing increases as cyst divisions progress resulting in 8-cell cysts with the most paired marks (Christophorou, Rubin, and Huynh, 2013). Chromosome pairing and double-stranded breaks (DSBs) must occur for meiosis initiation (Madigan, Chotkowski, and Glaser, 2002; Mehrotra and McKim, 2006). In early cysts, a protein-rich synaptonemal

complex (SC) forms between paired chromosomes, mediated by the protein C(3)G (Page and Hawley, 2001). This protein is an indicator of synaptonemal complex formation and can be visualized as early as 4-cell cysts and is dispersed through at least four cells as divisions continue. In the 16-cell cyst, the SC will only be maintained in the true oocyte (McKim et al., 2002; Lake and Hawley, 2012).

A critical step in oocyte fate is specifying the oocyte. Oocyte specification occurs in the last mitotic division, 8-cell cyst to 16-cell cyst, when one of two pro-oocytes exit meiosis and reverts to a nurse cell fate (King, 1970). The pro-oocyte that exits meiosis, becoming a nurse cell, will undergo cycles of endoreplication (repeated cycles of G1 and S phase). The other pro-oocyte remains arrested in prophase I of meiosis until stage 10 at which time it completes meiosis (McKim et al., 2002; Lake and Hawley, 2012).

Oocyte specification relies on oocyte-specific factors that are transcribed and translated during or directly after the last mitotic division. One oocyte-specific factor is a CPEB (cytoplasmic polyadenylation element binding) protein, oo18 RNA-binding protein (Orb). *Orb* and *gurken* (*grk*) are transcribed in the oocyte (Huynh and St Johnston, 2000; Navarro-Costa et al., 2016). *Orb* mRNA localizes around the fusome in 8-cell cysts and is localized to the one remaining pro-oocyte, the true oocyte, by 16-cell cysts (Cox and Spradling, 2003). The fusome also assists in timely and proper localization of proteins, mRNAs, and organelles to the pro-oocyte (Huynh and St Johnston, 2000; Lu et al., 2017). In mutants that have defects in oocyte specification, 16 nurse cells, and no oocyte, egg chambers can progress to stage 14 without an oocyte but are unviable (Mach and Lehmann, 1997; Huynh and St Johnston, 2000; Navarro et al., 2004).

Polarization of the oocyte is critical for oocyte fate. In 16-cell cysts in stage 2b of the germarium, the oocyte proteins anteriorly localize in the oocyte; however, in stage 1 (region 3 of the germarium) these proteins relocalize to the posterior of the oocyte (Figure 2A). This re-localization is complete by stage 3, and the oocyte remains posteriorly localized until another re-organization occurs in stage 9 (Theurkauf et al., 1992; de Cuevas and Spradling, 1998; Huynh and St. Johnston, 2000; Bolivar et al., 2001). For instance, *Bazooka* (*baz*), *PAR-1*, and *PAR-6* mutants accumulate oocyte-specific proteins in one cell that then never become localized to the posterior of the oocyte (Huynh et al., 2001a; Mach and Lehmann, 1997; Lantz et al., 1994). When the oocyte-specific factors fail to localize to the posterior of the oocyte, the cell expressing the oocyte factors exits meiosis and reverts to a nurse cell fate (Huynh et al., 2001a). Thus, *Baz*, *PAR-1*, and *PAR-6* mutants are required for polarization of oocyte-specific factors and to maintain the oocyte fate (Huynh et al., 2001b). Orb also helps to establish polarity in the oocyte (Huynh and St Johnston, 2004). Bicaudal-D (BicD) and Egalitarian (Egl), dynein regulatory proteins, are shuttled along with a network of polarized microtubules to the oocyte (Huynh and St Johnston, 2000). When Egl, BicD, and Orb proteins are absent or mislocalized, the oocyte is not maintained and reverts to a nurse cell fate (Mach and Lehmann, 1997; Huynh and St Johnston, 2000; Navarro et al., 2004). The oocyte undergoes re-localization again in stage 9 as the anterior-posterior (AP) and dorsal-ventral (DV) axes become established (Theurkauf et al., 1992; de Cuevas and Spradling, 1998; Huynh and St. Johnston, 2000; Bolivar et al., 2001; Roth, 2003). In stage 9, the localization of three mRNAs (*Bicoid*, *Oskar*, and *Gurken*) within the oocyte specifies the AP and DV axes (Riechmann and Ephrussi, 2001; van Eeden and St Johnston, 1999). *Bicoid* (*Bcd*) and *Oskar* (*Osk*) mRNAs localize to the anterior and

posterior poles, respectively (St Johnston and Nusslein-Volhard, 1992). The third mRNA, *Gurken* (*Grk*), defines the DV axis of the egg by localizing to the anterior-dorsal of the oocyte via indirect signaling from the follicle cells (Roth, 2003; Moussian and Roth, 2005). Along with the initial polarization of the oocyte within the germarium, this re-polarization in stage 9 is a key event that leads to egg and embryo axis formation.

The histone marks present in the euchromatin versus heterochromatin of the oocyte differ with development stage. For instance, histone 3 lysine 4 trimethylation (H3K4me3) is highly expressed in a transcriptionally active oocyte; whereas, by stage 4 of oogenesis oocyte compaction occurred, and the oocyte becomes transcriptional inactive. The oocyte does not express H3K4me3 in stage 4 of oogenesis (Navarro-Costa et al., 2016; Iovino, Ciabrelli and Cavalli, 2013). However, another histone mark, histone 3 lysine 27 trimethylation (H3K27me3), expressed highly in the oocyte at stage 4 of oogenesis (Navarro-Costa et al., 2016; Iovino, Ciabrelli and Cavalli, 2013). Epigenetic information such as histone marks is passed maternally from the mother and is used by the embryo for the first few hours of life.

One class of epigenetic factors that allows tight regulation of genes is known as chromatin modifiers. There are several different families of chromatin modifiers. A family of chromatin regulators called the Polycomb Group genes (PcG) are a family of readers that help to condense the chromatin so that it impedes transcription (Paro and Harte, 1996). One of the ways it does this is by adding repressive marks to the histones that help to promote the packing of the chromatin (Paro and Harte, 1996). In converse, in areas that need to be transcriptionally active, a second set of genes, the Trithorax (Trx) group, act as activators to change the methylation marks on the histones and recruit

chromatin remodelers to slide the nucleosomes apart, allowing RNA polymerase II access (Paro and Harte, 1996).

*Trithorax-like* protein (*Trl*), GAGA factor, is also a member of the Trx group and is a transcription factor that can block the repressive effects of histones and function in chromatin remodeling (Bhat et al., 1996). Evidence suggests that GAGA factor generates and maintains nucleosome-free regions by blocking inhibitory factors such as histone H1 (Bhat et al., 1996, Croston et al., 1991; Fuda et al., 2015). GAGA factor also has a role in recruiting RNA polymerase II by displacing nucleosomes at or near the promoter (Perez-Zamorano et al., 2017, Fuda et al., 2015). *Trl* regulates transcription of vital genes including those encoding heat-shock proteins (*hsp26*, *hsp27*, *hsp70*), homeotic and segmentation proteins (*Ubx*, *engrailed*, and *ftz*), ecdysone-inducible proteins (*E74*), and housekeeping proteins (*His3*,*His4*, *Actin 5C*, *α1-Tubulin*) (Soeller, Oh, and Kornberg, 1993; Gilmour, Thomas, and Elgin, 1989; Chung and Keller, 1990; O'Donnell and Wensink, 1994; Granok et al., 1995; Wilkins and Lis, 1997). The study of the *heat shock protein 26 (hsp26)* promoter indicates that GAGA generates and maintains nuclease hypersensitive regions (Bhat et al., 1996). Nuclease hypersensitive regions are short regions of DNA that have less compacted chromatin, allowing transcription factors and nucleases access to the DNA (Pennisi, 2012). Further, Trl maintains open regions of chromatin at regulatory regions to allow transcription factors access to bind (Perez-Zamorano et al., 2017).

*Trl* is involved in a multitude of developmental processes that may be aided by the domains and binding region flexibility of the protein produced. The Trl protein has 3 distinct domains: POZ/BTB, zinc finger, and glutamine-rich domains (Benyajati et al., 1997; Zollman et al., 1994). The POZ/BTB (Pox virus and Zinc finger/Broad-Complex,

Tramtrack, Bric a Brac) domain acts a protein interaction domain and mediates the formation of multimers (Benyajatin et al., 1997). There is little known about the role the glutamine-rich domain plays for the Trl protein. While zinc finger domains typically bind DNA, the zinc finger domain in Trl is unique in that there is only one $C_2H_2$ or finger whereas most genes that have a zinc finger domain have multiple fingers, $C_2H_2$ groups (Miller, McLachlan, and Klug, 1985). Trl binds a consensus pentamer, GAGAG, in target genes; however, the central G is the only nucleotide that must be conserved allowing flexibility of the nucleotides on either side of the G (O'Brien et al., 1995; Omichinski et al., 1997). Moreover, the *Trl* gene encodes ten known RNA isoforms that range in size from 66 to 120 kDa and exist due to alternative splicing (Figure 2) (Gilmour and Elgin, 1989; Benyajati et al., 1992). The function of the three domains, promiscuity of the binding sequence, and the existence of ten isoforms could be one explanation for how *Trl* is involved in so many different developmental processes.

*Trl* is essential for proper development of the *Drosophila* adult; *Trl* mutants cannot survive past larval stages, indicating fly development requires *Trl*. *Trl* plays an important role in the development of the *Drosophila* eye (Farkas et al., 1994; Dos-Santos et al., 2008), embryogenesis (Bhat et al., 1996), spermatogenesis (Dorogova et al., 2014) and oogenesis (Fedorova et al., 2018). Studies using a *Trl* hypomorph, $Trl^{13C}$, indicates *Trl* affects viability, females are sterile, and mutants die as embryos (Trunova et al., 2001; Farkas et al., 1994; Bhat et al., 1996). Another study using $Trl^{13C}$ mutants showed evidence of nuclear division defects indicating a possible role in the mitotic cell cycle (Bhat et al., 1996; Raff, Kellum, and Alberts, 1994). In another *Trl* hypomorph, $Trl^{s2325}$, mutants die at the third instar larval stage, and germ cell number reduction ranges from 8 to 14 germ cells in oogenesis (Trunova et al., 2001). $Trl^{R85}$ mutants were mostly sterile

with only 23 out of 807 laying a few eggs, but the eggs laid were unfertilized due to micropyle formation defects indicating as do other studies that *Trl* is required for oogenesis (Bejarano and Busturia, 2004). In oogenesis, $Trl^{362}$, $Trl^{en82}$, and $Trl^{(ex)15}$ global mutants have an abnormal number of oocytes, oocyte mislocalization, nurse cell defects, karyosome organization defects, and dorsal appendage formation defects (Fedorova et al., 2018; Omelina et al., 2011; Ogienko et al., 2006). The varying, large range of phenotypes associated with different *Trl* mutants suggests that the list of genes regulated by *Trl* is far from complete.

The oocyte defects in Trl global mutants could arise because of a loss of Trl activity specifically in germ cells or do to a non-autonomous function of Trl in somatic cells (Fedorova et al., 2018; Omelina et al., 2011; Ogienko et al., 2006). The autonomous effects of *Trl* in germ cells has not been studied. In this study, I hypothesize that *Trl* is necessary in the germline, specifically in the oocyte. In order to address this, I used *flippase* (*FLP)/FLP recognition target (FRT)* mediated recombination system to facilitate analysis of $Trl^{13C}$, $Trl^{s2325}$, and $Trl^{R85}$ homozygous mutant cells in the context of a wildtype ovary and a germline-specific *Trl RNAi* (Laws and Drummond-Barbosa, 2015).

## Methods

### *Fly Husbandry*

*Drosophila* stocks of all of the *Trl* mutants, wildtype (*yw*), and driver lines (*nosGal4*-NGT and *otuGal4*) were maintained at 22-25°C in vials with standard cornmeal/molasses/yeast/agar medium (NutriFly MF; Genesee Scientific) supplemented with yeast.

### *Genetic mosaic generation and stem cell analyses*

For genetic mosaic analyses using *flippase* (*FLP)/FLP recognition target (FRT),* the following alleles containing locus-appropriate *FRT* chromosomes were obtained: *hsFLP; armlacZ, neoFRT80B* (Bloomington Drosophila Stock Center (BDSC, #6341, virgins), $Trl^{s2325}$ (DGRC #111-392), $Trl^{13C(40)}$ (M. Frolov; Bayarmagnai et al., 2012), $y^{1};trc^{1}neoFRT80B\ ry^{506}\ kar^{2}/TM6C\ sb\ ry$ (BDSC, #5261, crossed with virgins as a control), *hsFLP; FRT79D Ubi-nGFP/TM3* (M. Buzczak; Buszczak and Segraves, 1998 virgins), $Trl^{R85}$ (A. Busturia; Bejarano and Busturia, 2004), w*; *P+mW.hs FRTwhs/2A* (BDSC, #1997, crossed with virgins as a control). Genetic mosaics were generated by *FLP/FRT*-mediated recombination in 2-3-day old females carrying a mutant allele alongside a wildtype allele (linked to an *armlacZ* or *GFP* marker) on homologous *FRT* arms, and a *hs-FLP* transgene (as described in Laws and Drummond-Barbosa, 2015). Flies were heat shocked at 37°C two times per day for 3 days and incubated at 25°C for 6-10 days with daily transfers to fresh yeast vials (standard media, initially supplemented with dry yeast, then supplemented with wet yeast paste on the last 3 days before dissection). $Trl^{s2325}$, $Trl^{13C(40)}$, and $Trl^{R85}$ mosaic germaria analysis occurred at 6, 8, and 10 days after clone induction. The generation of control mosaics used wild-type alleles.

GSCs were identified based on their fusome at the anterior portion of the cell and located at the junction with adjacent cap cells (de Cuevas and Spradling, 1998). Stem cell loss measurement was the percentage of GFP negative GSCs out of the total number of ovarioles (Laws and Drummond-Barbosa, 2015). Results were subjected to Chi-Square analysis using Microsoft Excel. Early germline cysts were identified based on fusome morphology (de Cuevas and Spradling, 1998; Spradling, 1993).

***RNAi generation and analyses***

For oocyte maintenance analyses in the germline, the *$y^1$ $v^1$; P{$y^{+t7.7}$ $v^{+t1.8}$=TRiP.GL00699}attP2* (BDSC, #41582, *Trl RNAi*) was crossed with the germline-specific driver *otuGal4* (T. Schupbach). Crossing *yw* (D. Drummond-Barbosa) flies with the *otuGal4* driver produced the driver control, and the RNAi control was the result of crossing *yw* flies with the *Trl RNAi*. For analyses of oocyte specification, the *Trl RNAi* was crossed with the germline-specific driver *nosGAL4-NGT* (referred to as *nos-Gal4*) (Rørth, 1998; Van Doren et al., 1998). Flies were collected one to two days after eclosion and maintained on standard medium supplemented with wet yeast paste (Drummond-Barbosa and Spradling, 2001) for six or ten days before ovary dissection.

***Immunofluorescence and microscopy***

Ovaries were prepared for immunofluorescence microscopy as described (Ables and Drummond-Barbosa, 2013). Ovaries were dissected and ovarioles teased apart in Grace's medium without additives (Lonza). After dissection, the ovaries were placed in pre-coated bovine serum albumin (BSA) tube and placed on ice. The ovaries were fixed in 5.3% formaldehyde in Grace's medium for 13 minutes at room temperature. The

ovaries were then washed 3-4 times for 30 minutes in phosphate-buffered saline (PBS, pH 7.4; Fisher) with 0.1% Triton X-100. Ovaries were permeabilized in 0.5% PBS-Triton for 30 minutes at room temperature and blocked for three hours in blocking solution [5% bovine serum albumin (Sigma), 5% normal goat serum (MP Biomedicals), and 0.1% Triton X-100 in PBS] at room temperature. Primary antibody dilution occurred in blocking solution and used overnight at 4°C: chicken anti-GFP (#13970, Abcam; 1:2000), chicken anti-B-gal (#9361, Abcam, 1:2000), rabbit anti-Trl (John T. Lis and Kevin White, 1:500), mouse anti-orb 4H8 and 6H4 (#42752, DSHB, 1:100 each), C(3)G (Mary Lilly, 1:5000), mouse anti-BicD (1B11 and 4C2) (#P16568, DSHB, 1:10 each), and rabbit anti-H3K4me3 (#491005, Life Technologies Corp, 1:200). Primary antibodies mouse anti-Hts(1B1) (Q02645, DSHB; 1:10) and mouse anti-Lamin C (LC28.26, DSHB; 1:100) incubated over two nights at 4°C. Following a two-hour incubation at room temperature with AlexaFluor 488-, 568- or 633-conjugated goat species-specific secondary antibodies (Life Technologies; 1:200). Ovaries were stained with 0.5 µg/ml 4'-6-diamidino-2-phenylindole (DAPI; Sigma) in 0.1% Triton X-100 in PBS and mounted in 90% glycerol mixed with 20% *n*-propyl gallate (Sigma). Confocal z-stacks (1 µm optical sections) were collected with a Zeiss LSM 700 laser scanning microscope using ZEN Black 2012 software. Images were analyzed using Zeiss ZEN Blue 2012 software, and minimally and equally enhanced via histogram using ZEN and Adobe Photoshop CS6.

## Results

### *Trl is highly expressed in the germline in 8-cell cysts through stage 8*

To begin to understand the function of *Trl* in oocyte development, I assessed Trl protein distribution in wildtype ovaries using a Trl-specific antibody (O'Brien et al., 1995; Sun et al., 2003). The epitope for the Trl-specific antibody is located at the carboxy-terminus of the Trl protein and is encoded at the 3' end of the *Trl* gene locus. (Figure 2) (O'Brien et al., 1995). I performed immunofluorescence staining on wildtype ovaries (Figure 1C-E). To determine which ovarian cells, express Trl, I used antibodies against the Adducin-like protein, Hu li tai shao (Hts; antibodies are also known as 1B1), which is prominently localized in germ cells (at the fusome) and in follicle cell plasma membranes (DSHB, #Q02645). Additionally, I localized the nuclear lamina protein LaminC, which is highly expressed in somatic cap cells (DSHB, LC28.26). To assess cellular localization, I co-stained ovaries with DAPI, a DNA marker. GSCs and CBs expressed low levels of Trl, but Trl levels dramatically increased in 8 and 16-cell cysts (Figure 1C, C', D, and D'). Trl antibody co-localized with the DNA in the nucleus of germ and somatic cells (Figure 1C-E). The nurse cells and the oocyte prominently expressed Trl until stage 7 and 8 (Figure 1E, E'). In stage 7, Trl expression decreased starting in the oocyte, and by stage 8, it was undetectable above background levels in the nurse cells.

### *Trl mutant germ cells do not have detectable Trl protein expression*

To test if the Trl antibody is specific, I stained *Trl* mutants previously described as genetic loss-of-function alleles. The *Trl* gene locus is complex, encoding 10 different RNA isoforms (Figure 2). The $Trl^{13C}$ and $Trl^{s2325}$ are P-element insertions that disrupt the large intron separating coding exons 1 and 2 of the *Trl* gene locus (Figure 2) (Spradling,

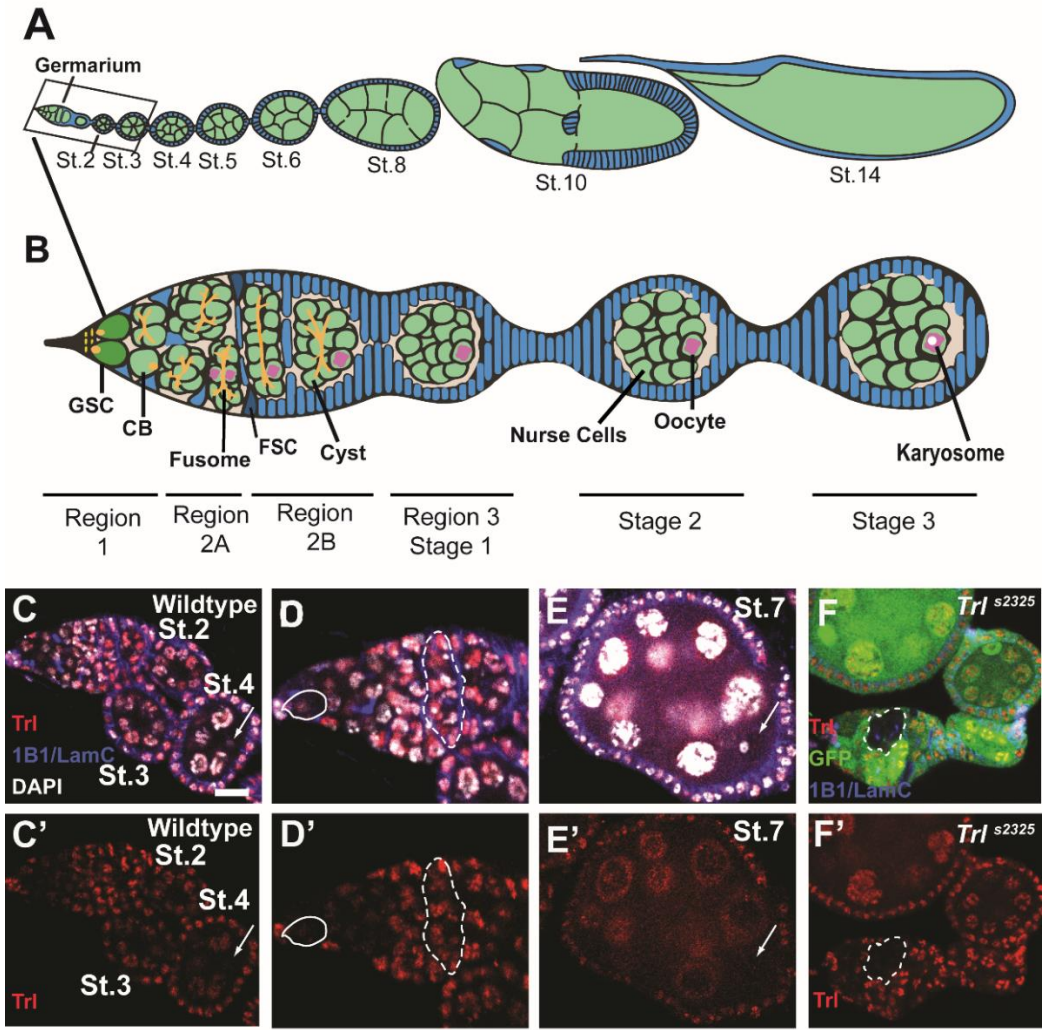


Figure 1: Trl protein is expressed in the germ cells from 16-cell cysts to stage 7.

The ovarioles are all oriented anterior on the left and posterior on the right. (A) A schematic of an ovariole with the germaria located at the most anterior tip. The germ cells are in green and the somatic cells are in blue. (B) The boxed area in A is zoomed in on B: showing the germarium, stage 2, and stage 3. Cap cells (yellow) are located at the most anterior tip of the germarium. Directly adjacent to the cap cells are the germline stem cells (GSCs) (dark green). The round and branched structure in the GSC, CB, and cysts is the fusome (yellow). Somatic cells are in blue; somatic cells include terminal filament, cap, escort, and follicle cells (Figure A, B). The follicle stem cells (FSC) (dark blue) give rise to all the follicle cells (light blue) after them. The oocyte (pink) is shown in each cyst. At stage 3, the karyosome has formed and is visible (white) in the oocyte nucleus. (C-E) Wildtype ovaries were stained with a Trl, 1B1, LamC, and DAPI antibodies. The germarium (C and D) and stages 2, 3, 4, (C) and 7 (E) are shown. A magnified view of the germarium from C is shown in D. The arrow indicates the oocyte in each picture. (D) The GSC is outlined by a solid white circle. A 16-cell cyst is outlined by a dotted white line. (C'-F') The panels on the bottom depict the Trl antibody expression pattern alone. (F) The FLP/FRT system was used to induce mutant clones in *Trl* $^{s2325}$ ovaries alongside wildtype cysts. (F) An 8-cell cyst as outlined by the dotted white line is directly next to a GFP+ wildtype 8 cell cyst. The *Trl* $^{s2325}$ ovaries were also stained with GFP, 1B1, and LamC antibodies. Scale bar is 10μm.

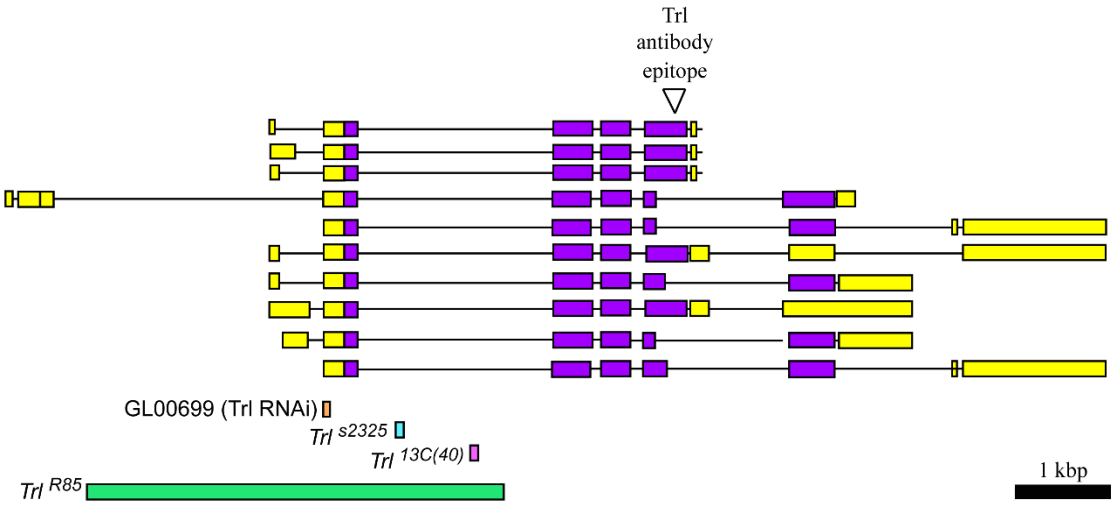


Figure 2: *Trl* gene locus and allele positions

The *Trl* gene schematic above is oriented 5' to 3'. There ten isoforms of the *Trl* gene each one is indicated in the schematic above. The yellow boxes depict non-coding regions. The purple boxes depict coding exons and the lines are the introns. Each allele used in this study is indicated by a different color underneath the gene schematic. The $Trl^{2325}$ (blue) and $Trl^{13C(40)}$ (pink) are both P-element insertions and the location of transposon insertion is being indicated by the boxes. The $Trl^{R85}$ allele is a deletion due to imprecise excision of the $Trl^{13C}$ transposon and removes a considerable region of the transcription unit indicated by the green box. The location targeted for the *Trl RNAi* is indicated by the orange box. The epitope for the Trl-specific antibody is located at the carboxy-terminus of the Trl protein and is encoded at the 3' end of the *Trl* gene locus. Scale bar is 1kbp.

1999; Trunova et al., 2001; Laney and Biggin, 1996). A null allele, *Trl* $^{R85}$, is a deletion resulting from imprecise excision of the *Trl* $^{13C}$ transposon that removes an extensive region of the *Trl* 5' transcription unit, including the transcription start site for all but one RNA isoform (Figure 2) (Farkas et al., 1994). Homozygous *Trl*$^{13C}$, *Trl* $^{s2325}$, and *Trl* $^{R85}$ mutants are not viable as adults (Farkas et al., 1994; Trunova et al., 2001; Laney and Biggin, 1996). I therefore generated mutant clones using the *flippase* (*FLP*)/*FLP recognition target* (*FRT*) mediated recombination system to facilitate analysis of *Trl*$^{13C}$, *Trl* $^{s2325}$, and *Trl* $^{R85}$ homozygous mutant cells in the context of a wildtype ovary (Laws and Drummond-Barbosa, 2015). In the *FLP/FRT* system, wildtype cells carry a ubiquitous Green Fluorescent Protein (GFP), while homozygous mutant clones lack GFP. The ovaries were stained with antibodies targeted against Trl, GFP (to distinguish mutant versus wildtype clones) and 1B1/LamC (fusome and membrane markers that allow me visualize germ cells). Trl protein is undetectable in the *Trl* $^{s2325}$ mutant 8-cell cysts (dotted white circle in Figure 1F, F'). In contrast, Trl protein is easily detected in similarly staged wildtype cysts. Therefore, I conclude that in both *Trl* alleles used in this study, full-length Trl protein was undetectable (Figure 1F-F'). Due to the location of the epitope (C' terminus) I cannot say for certain that these alleles are protein nulls because any Trl protein produced in these alleles likely lacks the C' terminus (Figure 2).

***Trl is not required for GSC maintenance***

The phenotype of the *Trl* global mutant ovaries includes an abnormal number of oocytes, oocyte mislocalization, nurse cell defects, karyosome organization defects, and dorsal appendage formation defects; however, these phenotypes could arise because of two possibilities: 1) loss of Trl activity specifically in germ cells, or 2) non-autonomous

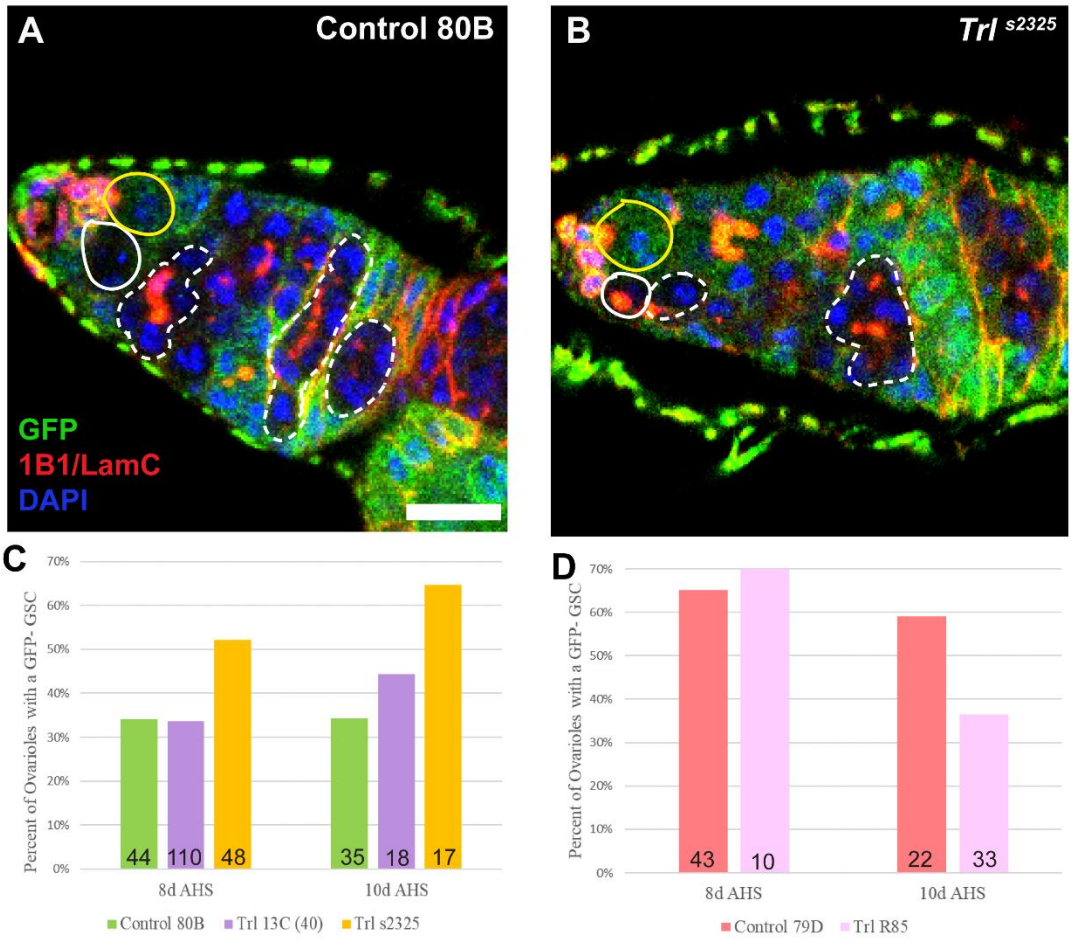


Figure 3: *Trl* is not required for GSC maintenance

Using the *FLP*/FRT system, wildtype and mutant cysts can be visualized alongside one another. (A) In the control, all cells are wildtype including GFP- cells. (B) Whereas in the mutant, wildtype cells are green (GFP+) while mutant clones lack GFP (GFP-). (A) The GFP- GSC, outlined in a solid white circle, has a corresponding GFP- 8 and 16-cell cysts, outlined by the dotted white circles. (A) The yellow solid circle shows a wildtype GSC. (B) In the *Trl* $^{s2325}$ mutant germarium, GFP-GSC mutant clones and GFP- mutant cysts are present, as indicated by the white solid and dotted circles respectively. Both germarium pictured in (A) and (B) were stained with GFP (green) to mark clones, 1B1 (red) to mark fusomes, LamC (red) to mark plasma and nuclear membranes, and DAPI, a DNA marker (blue). (C-D) A quantification was performed by calculating the number of ovarioles with a GFP-GSC over the total number of ovarioles counted for each control and mutant allele. This was conducted at two time points: 8 and 10 days after clone induction. Each allele is depicted in a different color on the graphs. A chi square test was run to determine whether each mutant allele (*Trl*$^{13C(40)}$ and *Trl* $^{s2325}$) is statistically different from the control (Control 80B) ($p<0.05$) (C). In D, a chi square test was run to determine whether *Trl* $^{R85}$ is statistically different from the control (Control 79D) ($p<0.05$) (D). Scale bar is 10μm.

function of Trl in somatic cells (Fedorova et al., 2018; Omelina et al., 2011; Ogienko et al., 2006). Oocytes arise from the activity of germline stem cells (GSCs) (Hanna and Hennebold, 2015). There are low Trl protein levels in GSCs and cystoblast (CB) which could still have an effect on GSCs. Thus, I first investigated whether the germline stem cells (GSCs) were affected by a loss of *Trl*. GSC proliferation and maintenance are essential for the development of an oocyte and eventual mature egg. Each time a GSC asymmetrically divides, a descendant of the CB will be an oocyte. GSCs have two fundamental characteristics: self-renewal and proliferation. The GSC divides producing two daughter cells: one will be retained within the niche as a GSC while the other will differentiate into a cystoblast. GSC maintenance is essential for gamete production. The *FLP/FRT* systems allows me to visualize mutant GSCs with their corresponding GFP- progeny alongside wildtype GSCs and their progeny. The ovaries were stained with antibodies targeted against GFP (to distinguish mutant versus wildtype clones), 1B1/LamC (fusome and membrane markers that allow me visualize germ cells), and DAPI, a DNA marker. GSC maintenance was measured by the number of GFP negative GSCs out of the total number of ovarioles. If the percent of GFP negative GSCs decreases significantly over time, then GSCs maintenance within the niche has not occurred. The percent of GFP- GSCs remained about the same in control ovaries (Control 80B) at 8 days after clone induction and at 10 days after clone induction (Figure 3A, C). The percent of GFP- GSCs also did not decrease significantly in the $Trl^{s2325}$ and $Trl^{13C\ (40)}$ mutant alleles (Figure 3B, C). In contrast, control ovaries (Control 79D) and $Trl^{R85}$ did show a non-significant decrease in percent of GFP-GSCs from 8 to 10 days after clone induction (Figure 3D). Altogether, I conclude that *Trl* mutants have negligible GSC loss (Figure 3C-D).

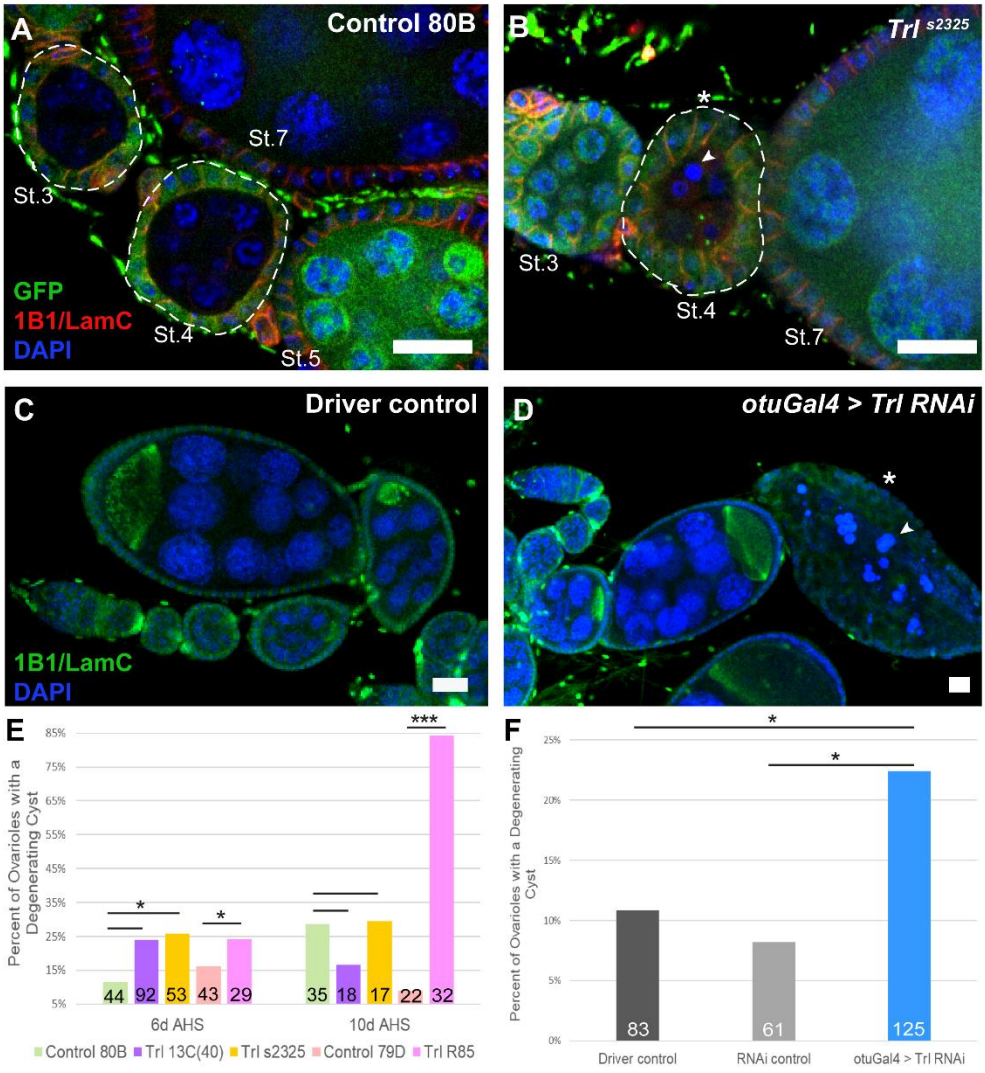


Figure 4: *Trl* is necessary for cyst survival

The *FLP/FRT* system was used to visualize wildtype and mutant cysts alongside one another. (A and B) The control and mutant ovaries were stained with GFP (green), 1B1(red), LamC (red), and DAPI (blue). (B) A degenerating cyst in *Trl* $^{s2325}$ with pyknotic nuclei is indicated with the asterisk. (E) A chi square test was run to determine whether each mutant allele (*Trl*$^{13C(40)}$, *Trl* $^{s2325}$, and *Trl* $^{R85}$) is statistically different from its corresponding control (Control 80B and Control 79D). (E) The lines above the bars indicate what is being compared, and the asterisks indicate that what is being compared is statistically significant ($p<0.05$. RNA interference (RNAi) using the otuGal4 driver was used to knockout *Trl* starting in the 16 cell cysts. (C and D) The control and *outGal4 > Trl RNAi* were both stained with 1B1 (green) (marks the fusome), LamC (green) (marks nuclear and plasma membranes), and DAPI (blue) (DNA marker). (F) A chi square test was run to determine whether the *otuGal4 > Trl RNAi* and each control (driver and RNAi) are statistically different from one another. (F) The lines above the bars indicate what is being compared, and the asterisks indicate that what is being compared is statistically significant ($p<0.05$). Scale bars are 10μm.

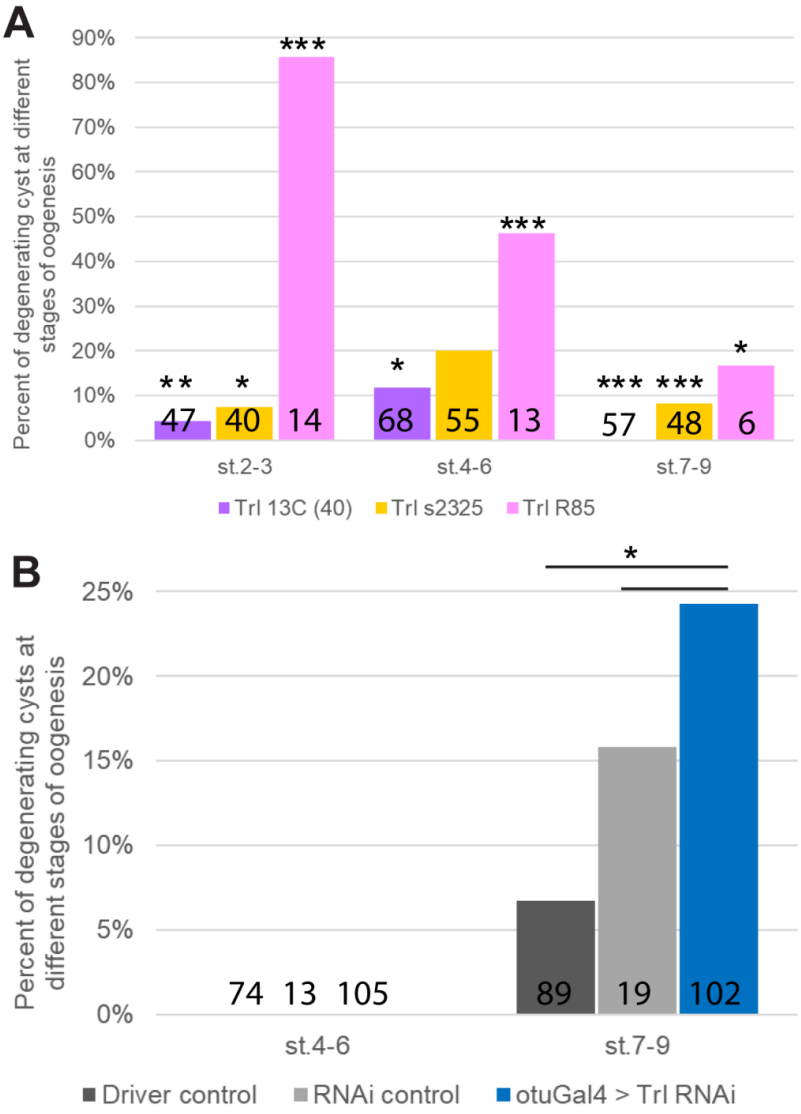


Figure 5: *Trl* cyst degeneration occurred at stages 2-4 and stages 7-9

(A) At 8 days after heat shock, the stage of each degenerating cyst in mosaic ovaries was determined. Cyst degeneration was determined by the size of the cyst and the stages of surrounding cysts. The cysts were placed into eight groups: st.1, st.2-3, st.4-6, st.7-9, and anything stage 10 and above. The total number of degenerating cysts were counted in each of these groups for both controls and mutant alleles. (A) A chi square test was run on each allele (shown) against its corresponding control (Control80B or Control79D; not shown). The asterisks indicate that the mutants are statistically different than the control at that stage of oogenesis (one asterisk – $p<0.05$; two asterisks – $p<0.001$; three asterisks – $p<0.0001$) (B) At 6 days after eclosion, the stage of each degenerating cyst in the RNAi ovaries was determined. The cysts were grouped similarly as in A. The total number of degenerating cysts were calculated in each of these groups for both controls and mutant alleles. (B) The lines above the bars indicate what is being compared, and the asterisk indicates that what is being compared is statistically significant ($p<0.05$).

***Trl mutant follicles undergo cyst degeneration at stages 2-4 and 7-9***

*Trl* global mutants undergo cyst death in stages 7-10 of oogenesis; however, the cyst death and location of the cyst death could arise because of a loss of Trl activity specifically in germ cells or due to a non-autonomous function of Trl in somatic cells (Fedorova et al., 2018). I also observed egg chambers that appeared to be dying when looking at the *Trl* mutant mosaics. In order to maintain cyst survival, only healthy cysts acquire the nutrients and resources needed. Cysts that are unnecessary or unhealthy are degraded in order to ensure resources are being used efficiently due to a high energy cost in producing mature eggs (Peterson et al., 2015). The *FLP/FRT* systems allows me to visualize germline mutant cysts (GFP-) alongside wildtype cysts (GFP+). The ovaries were stained with antibodies targeted against GFP (to distinguish mutant versus wildtype clones), 1B1/LamC (fusome and membrane markers that allow me visualize germ cells), and DAPI, a DNA marker. Cyst degeneration here is classified as a cyst that has highly condensed (pyknotic), fragmented nurse cell nuclei. In control ovaries (Control 80B), the nurse cell nuclei are polytene throughout the stages (Figure 4A); whereas, the *Trl* $^{s2325}$ germline mutant stage 4 clone has pyknotic nuclei (indicated by the arrowhead in Figure 4B). To test if *Trl* mutants have increased numbers of degenerating cysts, I recorded the total number of germline and mixed degenerating cysts out of the total number of ovarioles for each control and mutant allele. *Trl* $^{s2325}$ and *Trl* $^{R85}$ have statistically more cyst degeneration than their respective controls at 6 days after clone induction with p-values of 0.03 and 0.006 respectively (Control 80B and Control 79D respectively) ($p<0.05$) (Figure 4E). *Trl* $^{13C(40)}$ does not have statistically more cyst degeneration than the control with a p-value of 0.09 (Control 80B) ($p<0.05$) (Figure 4E). At 10 days after

with a p-value of $6.19 \times 10^{-7}$ ($p<0.0001$) (Figure 4E). clone induction, only *Trl* $^{R85}$ has statistically more cyst degeneration than the control 79D

RNA interference (RNAi) was used as another method to knock out *Trl* germline specifically. The germline-specific *Trl RNAi* knocked-down *Trl* in stage 2 of oogenesis using the *OtuGal4*. The ovaries were stained with antibodies targeted 1B1/LamC (fusome and membrane markers that allow me visualize germ cells) and DAPI, a DNA marker. The *Trl RNAi* revealed similar cyst degeneration as seen in the mosaics. In the driver control ovaries, the nurse cell nuclei are polytene (Figure 4C); whereas, the *OtuGal4 > Trl RNAi* stage 8 has pyknotic nuclei (indicated by the arrowhead in Figure 4D). The *OtuGal4 > Trl RNAi* has significantly more degenerating cysts than both controls ($p<0.05$) (Figure 4F).

Determining the stages in oogenesis where cyst degeneration occurs in the *Trl* mutants, allows for a better understanding of when *Trl* is needed for cyst survival. There are key checkpoints throughout oocyte development crucial to cyst survival. In wildtype ovaries, egg chamber degradation occurs at stage 7-9 when nutrients are limiting in order to maximize available resources (Pritchett et al., 2009; McCall, 2004). With this in mind, I hypothesized that *Trl* promotes cyst survival at the stage 7-9 nutritional checkpoint. To determine the stages where cyst degeneration occurs in *Trl* mutants, I counted the number of degenerating cysts at each stage of oogenesis over the total number of cysts at each stage. The stages in oogenesis were combined into four groups: stage 2-3, stage 4-6, stage 7-9, and stage 10+. In the absence of *Trl*, degeneration occurred most frequently at stages 2-4 in germline or mixed mosaic ovarioles (Figure 5A). *Trl*$^{13C\ (40)}$ and *Trl* $^{s2325}$ have their highest percentage of cyst degeneration (~12% and 20% respectively) at stage 4 of oogenesis (Figure 5A). The highest percentage of cyst degeneration (~85%) for the

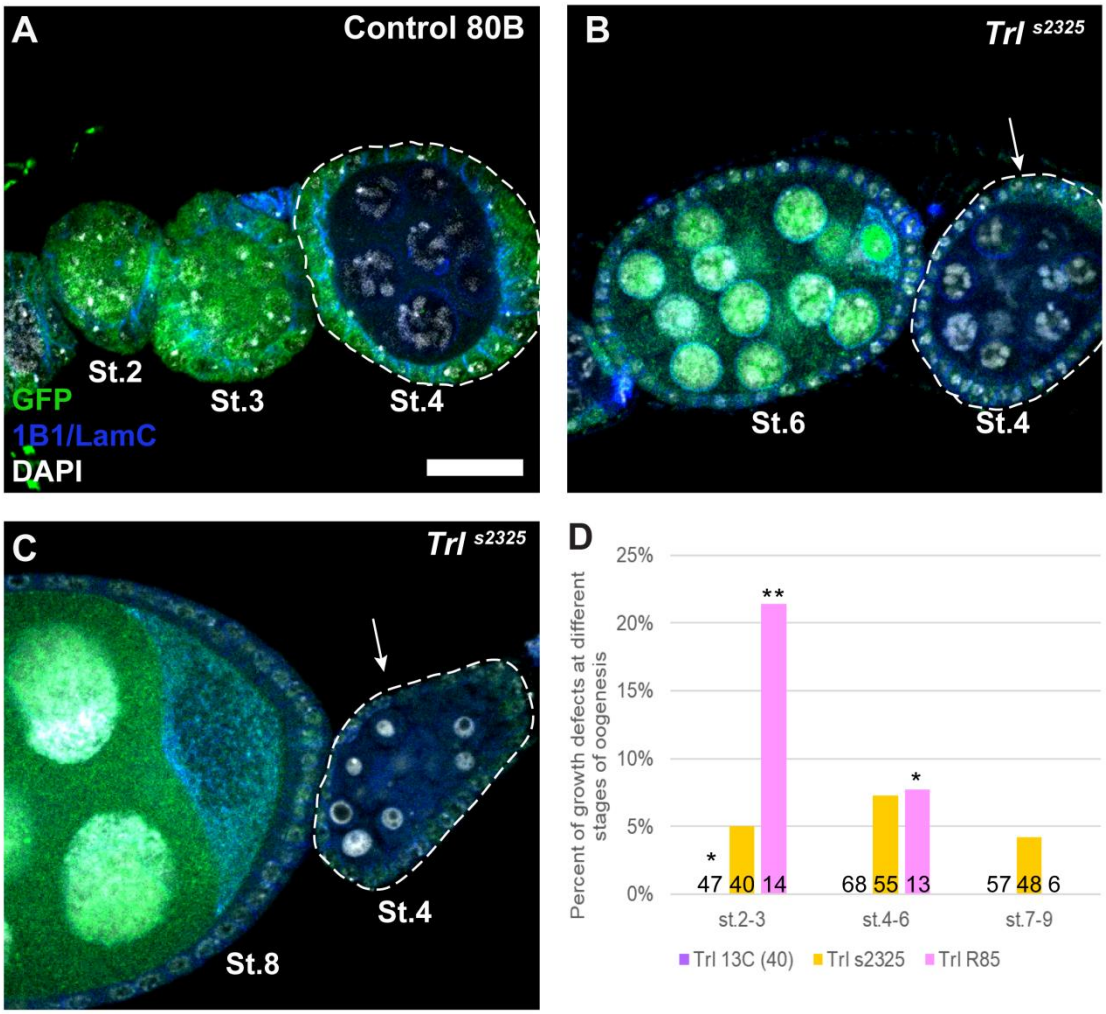


Figure 6: Growth defects occur between stages 2 and 3

(A-C) The *FLP/FRT* system was used in order to visualize wildtype and mutant clones alongside one another. (A-C) In both controls and mutant alleles growth defects, as determined by the presence of a smaller cyst following a larger cyst, occurred. Both the control and mutant ovaries were stained with GFP (green), 1B1(blue), LamC, (blue) and DAPI (white). Cyst stage was determined by size and surrounding cysts. (A) In Control 80B, a stage 2 and 3 is followed by a stage 4. (B and C) On two different instances, *Trl* $^{s2325}$ shows a stage 4 following a stage 6 and stage 7 respectively. (D) At 8 days after heat shock, the stage of each cyst with a growth defect was determined. The cysts were similarly grouped to previous experiments; however, everything in the germarium was grouped together instead of separately. The total number of growth defects were calculated in each of these groups for both controls and mutant alleles. A chi square test was run on each allele (shown) against its corresponding control (Control80B or Control79D; not shown). The asterisks indicated that the mutant is significantly different than the control at those stages in oogenesis (one asterisk – $p<0.05$; two asterisks – $p<0.001$). Scale bar is 10μm.

*Trl* $^{R85}$ allele occurred even earlier at stages 2 and 3 (Figure 5A). There were no degenerating mutant clones in stages 10-14. The germline-specific *Trl RNAi* knocked-down *Trl* in stage 2 of oogenesis using the *OtuGal4*. As quantified in the mosaics, the stage at which cyst degeneration occurred was also determined in the *Trl RNAi* and controls. Cyst degeneration was calculated by the number of degenerating cysts over the total number of cysts counted for each stage of oogenesis. The highest percentage of cyst degeneration (~24%) for the *OtuGal4 > Trl RNAi* occurred between stages 7-9. The *OtuGal4 > Trl RNAi* also had significantly more cyst degeneration at stages 7-9 than both controls ($p<0.05$) (Figure 5B). Taken together, my results demonstrate that *Trl* is essential in germ cells for egg chamber survival at stages 2-4 and 7-9.

***Trl may be necessary for proper cyst growth***

While imaging the mosaic samples, I observed defects in cyst growth. Defects in cyst growth indicate a separate developmental problem than just cyst survival defects alone. As a cyst progresses from the anterior to the posterior end of the ovariole, the cyst will grow and becomes elongated to accommodate the nurse cells as they undergo cycles of endoreplication. If a cyst cannot grow in size and elongate, then it could be targeted for programmed cell death to maximize resources for healthy cysts. I did not find any mention of a growth defect phenotype in the previous studies conducted with *Trl* mutants. A growth defect is defined here as a smaller cyst that follows a larger cyst; for instance, if stage 4 comes after stage 6. A cyst growth defect would likely only be detectable using the *FLP/FRT* system because *Trl* is being knocked down only in particular cell types. In contrast in global mutants, in which all cells are mutant, growth defects would be undetectable as all the egg chambers would be affected and would grow more slowly. To

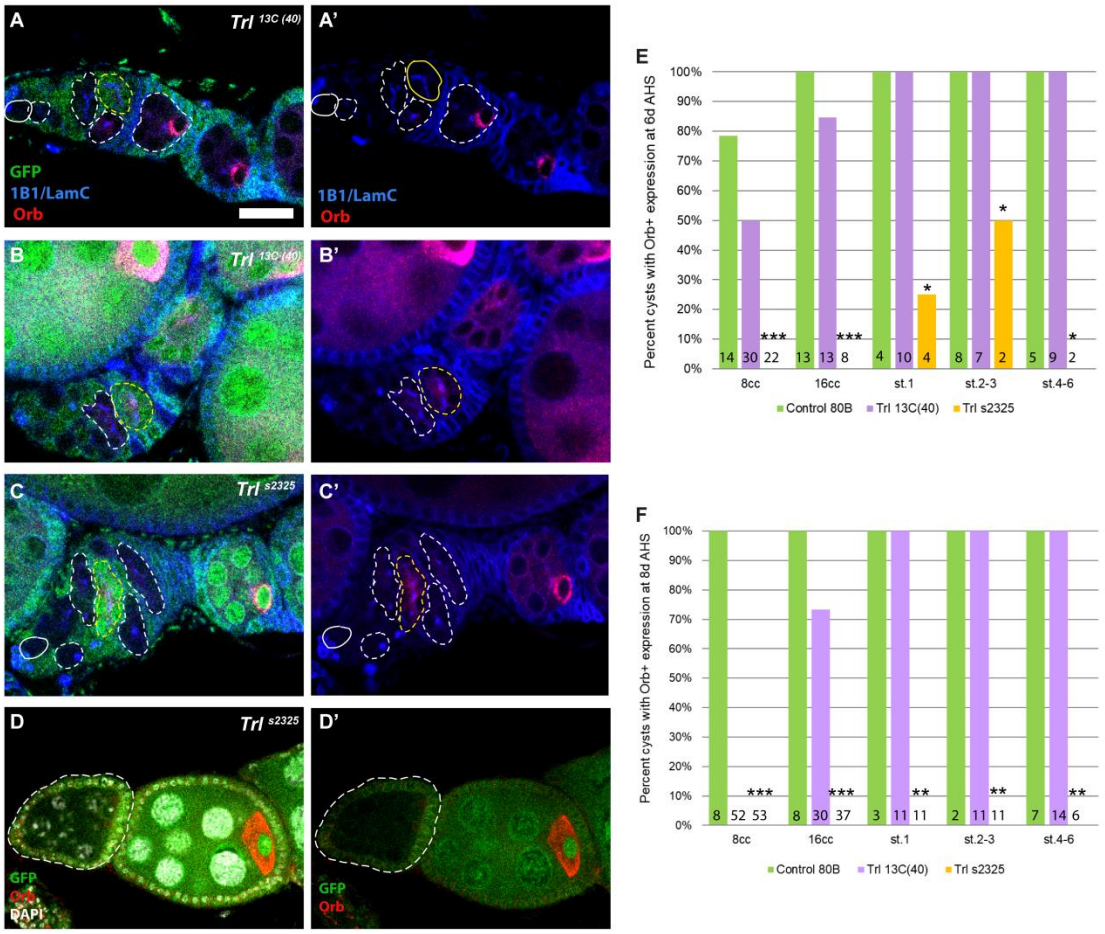


Figure 7: *Trl* is necessary for orb in the oocyte

The *FLP/FRT* system was used in order to visualize wildtype and mutant clones alongside one another. Mutant cysts are outlined by a dotted white circle while wildtype cysts are outlined by a dotted yellow circle. (A, A', B, B', E, and F) The *Trl* $^{13C(40)}$ mutant cyst (GFP-) had a delay in *Orb* expression. (C, C', D, and D') *Trl* $^{s2325}$ mutants at 8 days after clone induction illustrate that *Orb* is not expressed throughout all stages in which mutant clones were observed. (E) However, at 6 days after clone induction there were a small number of *Trl* $^{s2325}$ mutant cysts that had *Orb* expression. (A-B, E, and F) When the percent of cysts with Orb+ expression was calculated at both 6 and 8 days after clone induction the delay in *Trl* $^{13C(40)}$ can be seen more clearly. The ovarioles in A-C were stained with GFP (green), 1B1 (blue), LamC (blue), and Orb (red; a marker for the oocyte); A'-C' shows 1B1, LamC, and Orb. The ovariole in D was stained with GFP (green), Orb (red), and DAPI (white); while D' only shows GFP and Orb. (C-D, E, and F). Scale bar is 10μm.

test if *Trl* functions in germ cells to promote cyst growth, I counted the number of GFP- cyst growth defects at each stage of oogenesis out of the total number of germline and mixed cysts at each stage. In a wildtype ovariole, stage 3 would precede a stage 4 cyst (Figure 6A). However, in the *Trl* $^{s2325}$ mutant ovarioles, there is a stage 4 following stage 6 and 8 (Figure 6B and C respectively). The highest percent of cyst growth defects (~7%) in *Trl*$^{s2325}$ mutant clones occurred at stage 4 of oogenesis (Figure 6D). *Trl* $^{R85}$ mutant cyst defects occur earlier in stages 2 and 3 (~22%) (Figure 6D). However, *Trl* $^{13C\ (40)}$ had no observable growth defects at any stage of oogenesis (Figure 6D). Altogether this data suggests that *Trl* is required in germ cells for follicle growth. I cannot rule out that the cyst growth phenotype is independent of cyst death in this assay, because both occur at the same stages of oogenesis.

***Trl is necessary for Orb expression in the oocyte***

The phenotype of *Trl*$^{362}$ global mutant oocytes includes an abnormal number of oocytes and oocyte mislocalization; however, these phenotypes could arise because of a loss of Trl activity specifically in germ cells or due to a non-autonomous function of Trl in somatic cells (Ogienko et al., 2006). The production of viable egg chambers relies on the formation and maintenance of an oocyte (Iida and Lilly, 2004). I next looked at whether the oocyte was affected by a loss of *Trl*. In the literature, *Orb* is used as a marker for the oocyte. *Orb*, an RNA-binding protein, is produced in the oocyte and can first be detected at the fusome in 8 and 16-cell cysts in region 2a and 2b of the germarium (Huynh and St Johnston, 2000; Navarro-Costa et al., 2016). I hypothesized that *Trl* would be required for *Orb* in the oocyte. The *FLP/FRT* systems allows me to visualize germline mutant cysts (GFP-) alongside wildtype cysts (GFP+). The control and mutant ovaries

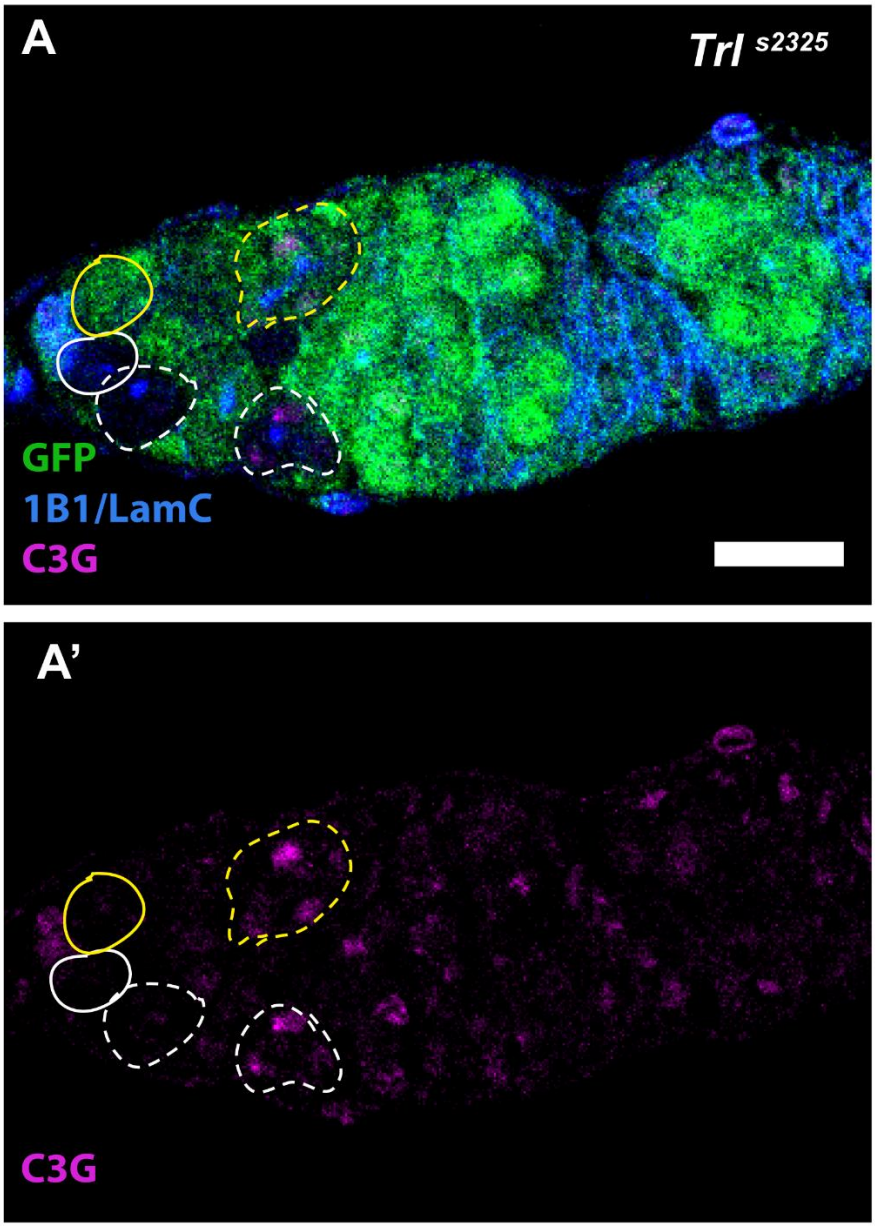


Figure 8: *Trl* is not necessary for synaptonemal complex formation

Immunofluorescence staining was performed on *Trl*$^{s2325}$ mosaic ovaries. Synaptonemal complex formation is marked using the C(3)G antibody (magenta). The samples were also stained with GFP (green), to mark clones, and 1B1/LamC (blue), to mark the fusome and plasma and nuclear membranes. *Trl*$^{s2325}$ mutant GSC and cyst are outline with white solid and dotted circles respectively. Wildtype GSC and cyst are outline with yellow solid and dotted circles. Scale bar is 10μm.

were stained with antibodies targeted against GFP (to distinguish mutant versus wildtype clones), 1B1/LamC (fusome and membrane markers that allow me visualize germ cells), Orb (the oocyte RNA-binding protein) and DAPI, a DNA marker (Figure 7A-D). To test if *Trl* is necessary in the oocyte for proper *Orb* accumulation, I counted the number of *Orb* positive and *Orb* negative clones at each stage in oogenesis out of the total number of cysts counted at each stage of oogenesis. The data was combined into five subgroups: 8-cell cysts (8cc), 16-cell cysts (16cc), stage 1, stage 2-3, and stage 4-6. $Trl^{13C\ (40)}$ mutant clones have a delay in *Orb* expression at both 6 and 8 days after clone induction (Figure 7A, A', B, B,' E, and F). The delay in $Trl^{13C\ (40)}$ mutants becomes more dramatic at 8 days after clone induction as seen by the complete lack of *Orb* expression in 8-cell cysts (Figure 7F). In $Trl^{13C\ (40)}$ mutant 16-cell cysts (8 days after clone induction), 75% have *Orb* expression, and by stage 1 within the germarium, 100% of the mutant clones express *Orb* (Figure 7F). In contrast, $Trl^{s2325}$ mutants only have a small number of cysts that are *Orb* positive at 6 days after clone induction (Figure 7E) and by 8 days after heat shock, $Trl^{s2325}$ clones never express the oocyte marker, *Orb* (Figure 7C, D, and F). Altogether, I conclude that *Trl* is necessary in the oocyte for proper accumulation of *Orb*.

***Trl is not necessary for entry into meiosis***

In order to understand if the role of *Trl* in the oocyte is limited to its effect on *Orb* accumulation, I next looked at whether entry into meiosis was affected by a loss of *Trl*. Oocyte determination relies on the oocyte entering meiosis while the nurse cells undergo endoreplication. Chromosome pairing and double-stranded breaks must occur prior to entry into meiosis (Madigan, Chotkowski, and Glaser, 2002; Mehrotra and McKim, 2006). In early cysts, a protein-rich synaptonemal complex (SC) forms between paired

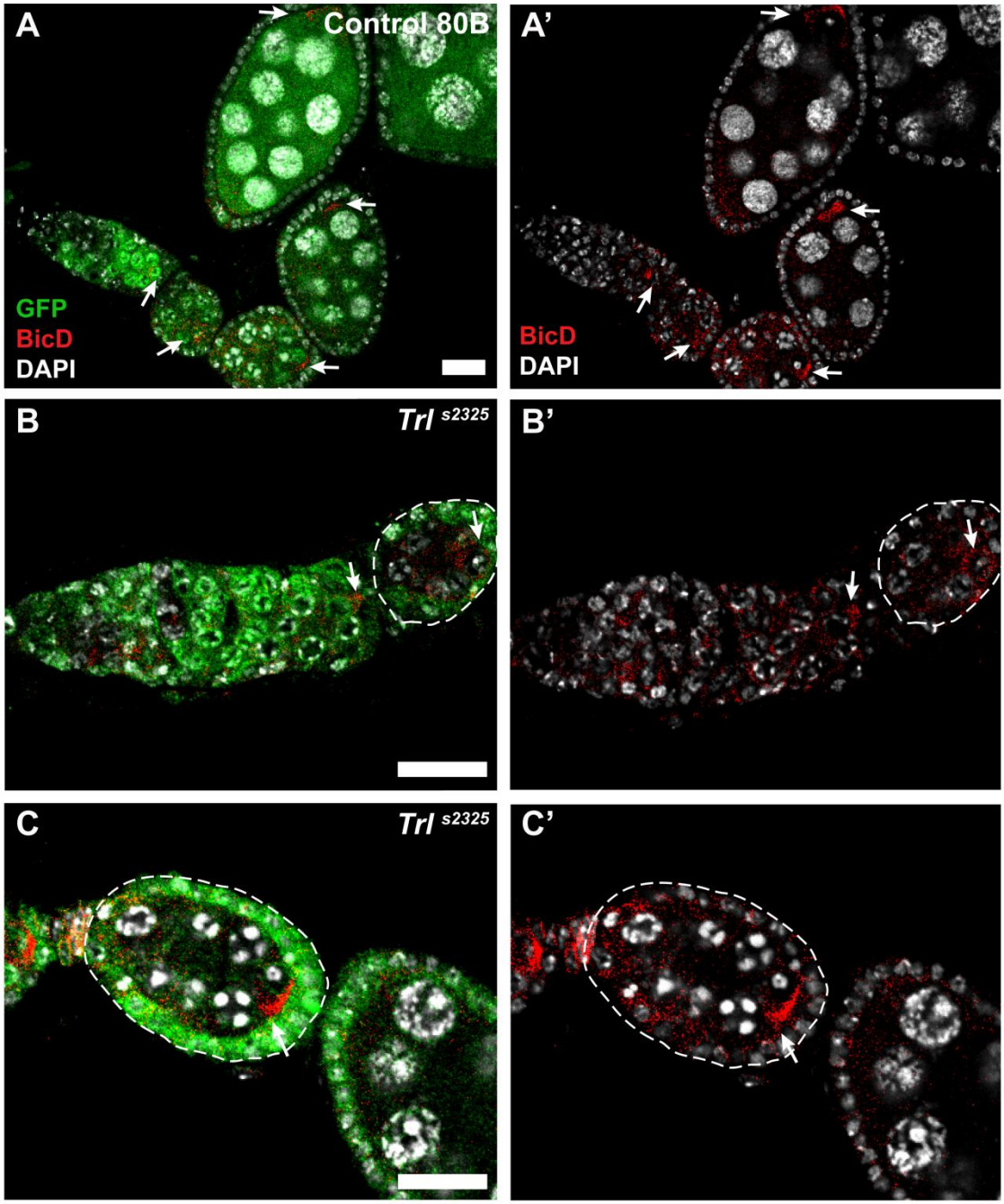


Figure 9: *Trl* is not necessary for oocyte polarity

Immunofluorescence staining was performed on control and *Trl*$^{s2325}$ ovaries. The ovaries were stained with BicD, an oocyte-specific factor made by the nurse cells (red). (A-C) The first column is stained with GFP (green), BicD (red), and DAPI (white). (A'-C') The second column shows the same ovarioles pictured in A-C however only BicD and DAPI are shown. (A-A') Control and (B, B', C and C') (B, B', C, C') *Trl*$^{s2325}$ mutant clones are circled by a dotted white line. (A-C, A'-C') The white arrows indicate *BicD* expression in the cysts. Scale bars are 10μm.

chromosomes mediated by the protein C (3)G (Page and Hawley, 2001). This protein, as an indicator of SC formation, first becomes visible as early as 4-cell cysts and disperses through at least 4 cells as divisions continue (McKim et al., 2002; Lake and Hawley, 2012). The *FLP/FRT* systems allows me to visualize germline mutant cysts (GFP-) alongside wildtype cysts (GFP+). The control and *Trl* $^{s2325}$ mutant ovaries were stained with antibodies targeted against GFP (to distinguish mutant versus wildtype clones), 1B1/LamC (fusome and membrane markers that allow me visualize germ cells) and C(3)G (SC formation marker) (Figure 8A-B).To test if the SC forms properly in *Trl* mutants, I counted the number of mutants clones in the germarium that were C(3)G+ out of the total number of mutant clones counted. The percentage of cysts that were C(3)G+ was also calculated in wild-type cysts within the germarium. *Trl* $^{s2325}$ mutants form a synaptonemal complex in all mutant clones observed (100% were C (3)G+) (Figure 8A, B). When compared to wildtype cysts of the same stage, the intensity and timing of SC formation did not differ (Figure 8A, B). From this, I can conclude that *Trl* is not necessary for entry into meiosis.

***Trl is not necessary for oocyte or cyst polarity***

The phenotype of *Trl*$^{362}$ global mutant oocytes includes an abnormal number of oocytes and oocyte mislocalization; however, these phenotypes could arise because of a loss of Trl activity specifically in germ cells or due to a non-autonomous function of Trl in somatic cells (Ogienko et al., 2006). Oocyte polarization is essential for maintaining oocyte fate. Early polarization of the oocyte disruption occurs when any of the *PAR* proteins are mutated, and maintenance of oocyte fate fails to occur (Benton, Palacios, and St Johnston, 2002; Cox et al., 2001; Huynh et al., 2001). The *FLP/FRT* systems allows

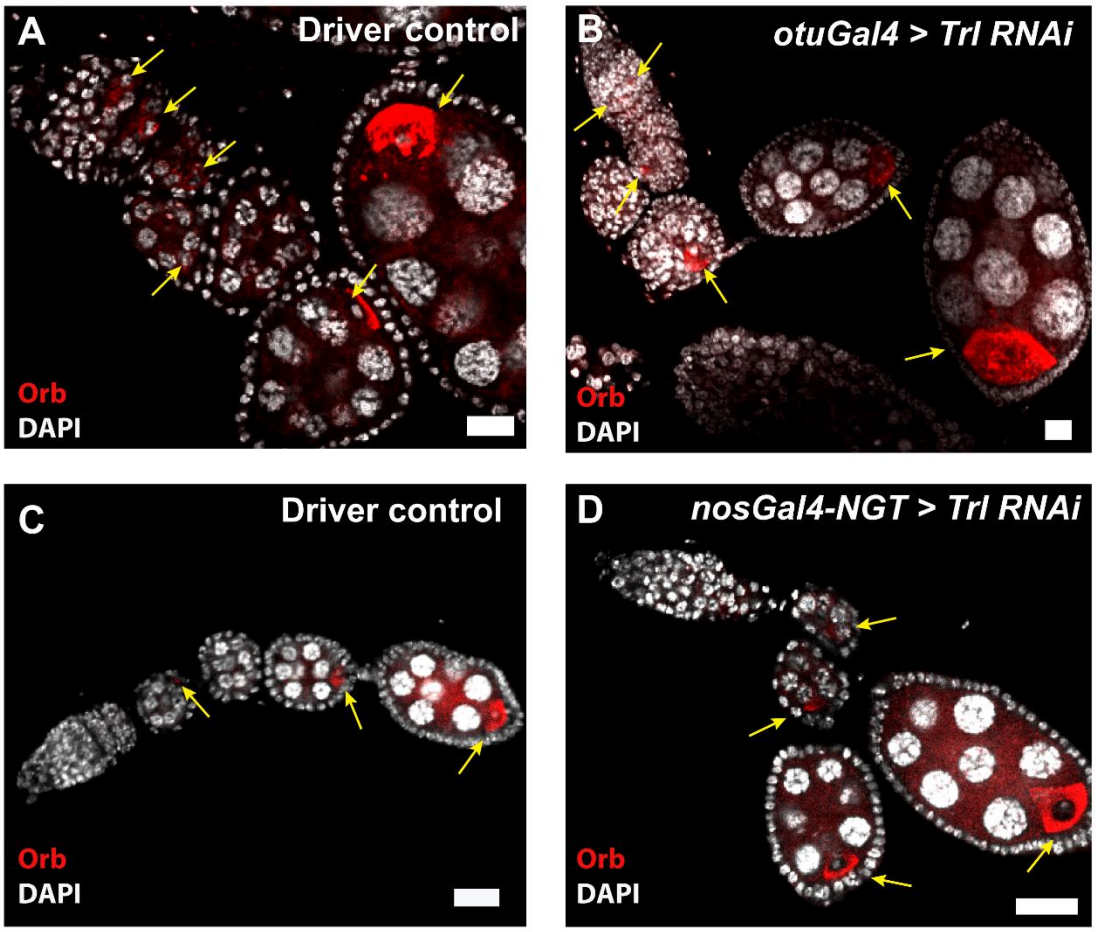


Figure 10: *Trl* is not required for specification or maintenance of the oocyte.

RNAi was performed using the otuGal4 (knocks out expression of transgene starting in 16 cell cyst) and nosGal4-NGT (knocks out expression of transgene starting in the GSC and CB) drivers. (A-D) The control and mutant ovaries were stained with Orb (red) and DAPI (white). (A and B) The *otuGal4 > Trl RNAi* ovarioles showed *Orb* expression starting in the 8-cell cyst and continuing throughout into late stages which is not different than *Orb* expression in the controls. (C and D) The *nosGal4-NGT > Trl RNAi* illustrates a similar *Orb* expression pattern between the controls and *Trl RNAi* as seen in the otuGal4 ovarioles. (C and D) *Orb* begins expressing in 8-cell cysts and continues to be expressed into late stage egg chambers. The yellow arrows indicate the oocyte. Scale bars are 10μm.

me to visualize the oocyte in germline mutant cysts (GFP-) alongside the oocyte in wildtype cysts (GFP+). The control and *Trl* $^{s2325}$ mutant ovaries were stained with antibodies targeted against GFP (to distinguish mutant versus wildtype clones) and DAPI, a DNA marker. Ovaries were co-stained with BicD, an oocyte-specific factor required for oocyte polarity that is localized to the posterior pole of the oocyte (Theurkauf et al., 1992; de Cuevas and Spradling, 1998; Huynh and St. Johnston, 2000; Bolivar et al., 2001). In both control (Control 80B) and *Trl* $^{s2325}$ mutant clones, the oocyte is posteriorly localized within the cyst. In the control, *BicD* expression is detectable and localizes to the posterior side of the oocyte in stages 1-8 (Figure 9A, A'). *Trl* $^{s2325}$ clones at stage 3 (B, B') and stage 6 (C, C') express *BicD* on the posterior side of the oocyte (Figure 9). *Trl* mutant cysts do not differ from the control in that they have normal polarity and express the oocyte marker, *BicD*, in stages 1-8. From this data, I conclude that *Trl* is not necessary for oocyte or cyst polarization.

***Trl is not required for oocyte maintenance***

I now know that *Trl* is not required for entry into meiosis or for oocyte and cyst polarity, but *Trl* is necessary for Orb accumulation in the oocyte. In order to understand the timing of when *Trl* is necessary for *Orb* accumulation in the oocyte, I investigated whether *Trl* is required for oocyte maintenance. Oocyte maintenance is determined after oocyte specification occurs and is indicative of a sustained oocyte in early and late stage cysts. If the oocyte reverts to a nurse cell fate after oocyte specification, then the oocyte's fate has not been maintained. Specification of the oocyte occurs in the last mitotic division from 8-cell cyst to 16-cell cyst. At this time, one of the pro-oocytes will exit meiosis, reverting to a nurse cell fate, while the other remains in meiosis and becomes the

oocyte (King, 1970). *Bazooka (Baz), PAR-1* and *PAR-6* mutants give rise to cysts that have 16 nurse cells and no oocyte much like *BicD* and *Egl* mutants (Huynh et al., 2001a; Mach and Lehmann, 1997; Lantz et al., 1994). In *BicD* and *egl* mutants, oocyte-specific proteins, such *Orb*, are not restricted to one cell; however, *Baz, PAR-1,* and *PAR-6* accumulate oocyte-specific proteins in one cell that then never become localized to the posterior of the oocyte (Huynh et al., 2001a; Mach and Lehmann, 1997; Lantz et al., 1994). In these mutants, when the oocyte-specific factors and centrosomes fail to localize to the posterior of the oocyte the cell expressing the oocyte factors exits meiosis and reverts to a nurse cell fate (Huynh et al., 2001a). Thus, *Baz*, *PAR-1*, and *PAR-6* mutants are not required for oocyte specification but are required to maintain the oocyte fate (Huynh et al., 2001b). Oocyte maintenance was investigated using two different germline-specific RNAi drivers (*OtuGal4* and *NosGal4-NGT*) that knock out transgene expression at different times in the germarium. In both experiments, the ovaries were co-stained with Orb (oocyte RNA-binding protein) and DAPI (DNA marker). I started with the *OtuGal4* driver which knocks out the gene of interest starting in 16-cell cysts; however, in the *OtuGal4 > Trl RNAi*, Trl protein expression is not effectively knocked out until stage 2 of oogenesis. The *OtuGal4 > Trl RNAi,* has Orb expression starting in 8-cell cysts and continues to be expressed throughout the ovariole (Figure 10A-B). The expression pattern in the *OtuGal4 > Trl RNAi* is not significantly different from Orb expression seen in the controls. I can conclude that *Trl* is needed before stage 2 from the *OtuGal4 > Trl RNAi*. The *NosGal4-NGT*, was used next to tease apart the timing of when *Trl* is needed for oocyte development. The *NosGal4-NGT* driver knocks out gene expression in the germline starting in late mitotically dividing cysts (8 and 16-cell cysts); however, the stage when Trl protein is effectively degraded is unknown. The *NosGal4-*

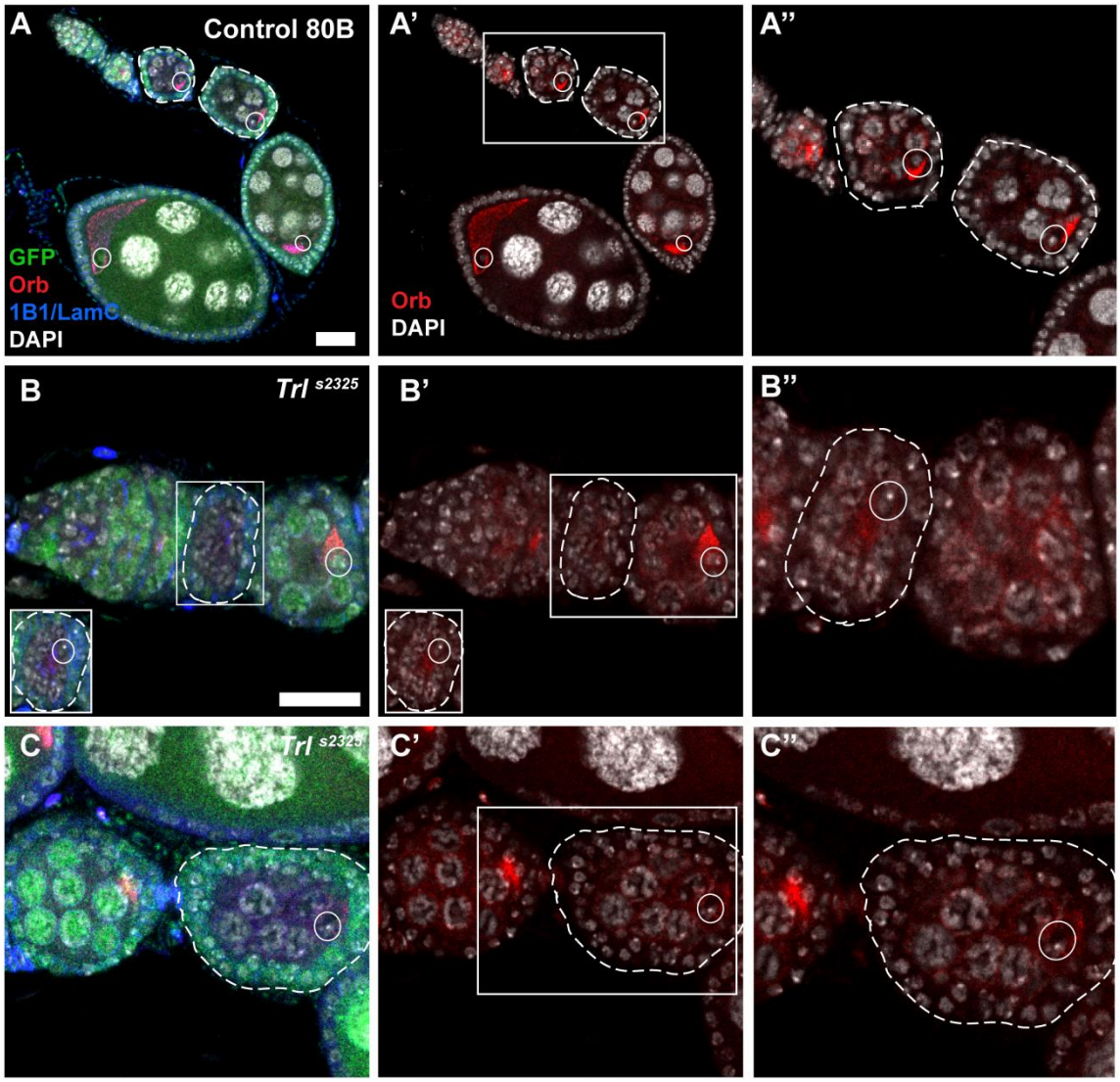


Figure 11: Oocyte has a condensed nucleus in *Trl* mutants

The *FLP/FRT* system was used to create homozygous mutant cysts (GFP-) directly beside homozygous wildtype cysts (GFP+). As a reminder in the control, all cells are wildtype even GFP- clones. (A-C) The control and mutant ovaries were stained with GFP (green), 1B1(blue), LamC (blue), Orb (red), and DAPI (white). In order to visualize the *Orb* expression, A'-C' only have Orb and DAPI staining on them. A zoomed in view of the boxed region in each picture is shown in A''-C''. *Trl* mutants such as *Trl* $^{s2325}$ have condensed oocyte nuclei even in the absence of *Orb* expression (B, B', B''C, C', C''). These condensed oocyte nuclei do not appear any different than that seen in the control (A, A', A''). Scale bars are 10μm.

*NGT >Trl RNAi,* has *Orb* expression starting in 8-cell cysts and continues throughout the rest of the ovariole. The *Orb* expression pattern seen in the *NosGal4-NGT >Trl RNAi* was not significantly different from the controls (Figure 10C-D). I can conclude from both the *OtuGal4 > Trl RNAi* and *NosGal4-NGT >Trl RNAi* data that *Trl* is not necessary for oocyte maintenance, and *Trl* is required before stage 2 for *Orb* accumulation.

### *Trl* mutants have a condensed oocyte nucleus

Typically, when a mutant lacks Orb expression there are 16 nurse cells and no oocyte (Mach and Lehmann, 1997; Huynh and St Johnston, 2000; Navarro et al., 2004). When looking at Orb in my mosaics, I noticed that there was a cell that appeared to be an oocyte despite a lack of *Orb* accumulation. Nurse cell DNA morphology is polyploidy while the oocyte has tightly compacted DNA, making them distinguishable from one another. If the oocyte in my *Trl* mutants had reverted to a nurse cell fate, I would expect to find 16 polyploid cells. In *Orb*, *BicD*, and *Egl* mutants, the oocyte fails to express oocyte markers and the DNA morphology is indistinguishable from nurse cells (Mach and Lehmann, 1997; Huynh and St Johnston, 2000; Navarro et al., 2004). The *FLP/FRT* systems allows me to visualize the DNA of cells in germline mutant cysts (GFP-) alongside the DNA of cells in wildtype cysts (GFP+). The control and *Trl* $^{s2325}$ mutant ovaries were stained with antibodies targeted against GFP (to distinguish mutant versus wildtype clones) Orb (an oocyte RNA binding protein) and DAPI, a DNA marker. To test if chromatin compaction in the oocyte is normal in *Trl* mutants, I used DNA morphology as an indicator of polyploidy versus tightly compacted DNA. As expected, the control ovaries have a compacted oocyte nucleus in each cyst (Figure 11A, A', A''). In *Trl*$^{s2325}$ mutant cysts, the oocyte nucleus has compacted DNA similar to the control

(Figure 11A-C). I conclude that chromatin compaction in the oocyte does not require *Trl*, and despite a lack of *Orb* oocytes are produced in the absence of *Trl*.

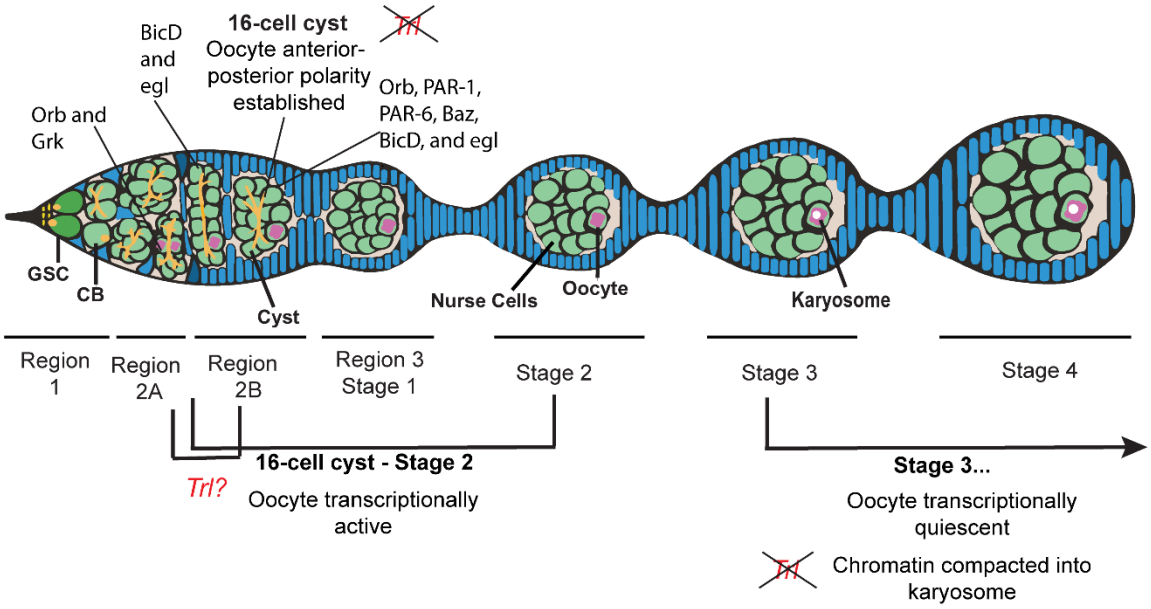


Figure 12: Model of the role *Trl* plays in early oocyte fate

The model depicts early oocyte development and based on the results of this study the role *Trl* plays in oocyte development. *Trl* with an X over it indicates that *Trl* is not required for those aspects of oocyte fate. For instance, *Trl* is not required for chromatin compaction in the oocyte beginning at stage 3 of oogenesis. This model also indicates when oocyte-specific factors are transcribed and translated. *Orb* and *Grk* are transcribed and translated by the oocyte in 8-cell cysts. Whereas, *BicD* and *Egl* are not transcribed and translated by the nurse cells until the lens-shaped 16-cell cyst. Oocye anterior-posterior polarity is established when the 16-cell cyst goes from lens-shaped to more round. At this time, *Orb*, *PAR-1*, *PAR-6*, *Baz*, *BicD*, and *Egl* are polarized to the posterior of the oocyte. The subset bracket within the oocyte transcriptionally active region is the proposed area in which *Trl* is necessary.

## Discussion

In this study, I investigated the effects of *Trl* in the germline during oogenesis. I hypothesized that *Trl* is necessary in the germline specifically for oocyte function. This study concludes that *Trl* is not required for establishing oocyte fate but is specifically necessary in oocytes for the accumulation of the RNA binding protein *Orb*.

The epitope of the Trl-specific antibody is located on the C' terminus of the Trl protein and is encoded at the 3' end of the gene locus (Figure 2). The C' terminus was chosen to avoid cross-reactivity because the BTB/POZ domain in the N' terminus is homologous to transcription factors *Broad Complex* and *tramtrack* (Soeler et al., 1993 and Bardwell and Treisman, 1994). $Trl^{13C\,(40)}$ and $Trl^{\,s2325}$ are P-element insertions that disrupt the large intron separating coding exons 1 and 2 of the *Trl* gene locus (Figure 2) (Spradling, 1999; Trunova et al., 2001; Laney and Biggin, 1996). Due to the location of the epitope for the *Trl* antibody, it cannot be concluded that $Trl^{13C(40)}$ and $Trl^{s2325}$ are protein nulls.

### *Trl is not necessary for GSC maintenance in oogenesis.*

My data suggests that *Trl* is not necessary in GSCs for their maintenance. Due to low Trl protein levels in GSCs, *Trl* had the potential to influence GSCs; however, the sample sizes at both 8 and 10 days after clone induction were small only enabling me to conclude negligible GSC loss (Figure 1 and Figure 3). *Trl* was shown to be required for primordial germ cell (PGC) progression into mature sperm in spermatogenesis (Dorogova et al., 2014). However, there was no evidence in the literature to suggest that *Trl* was necessary for GSC maintenance in oogenesis.

***Cyst degeneration in Trl mutants correspond to key checkpoints in oocyte development***

In *Trl* mutants, cyst degeneration occurred in stages 2-4 and 7-9. $Trl^{s2325}$ and $Trl^{R85}$ both had significantly more cyst degeneration that the controls while $Trl^{13C\ (40)}$ did not have significantly more cyst degeneration that the control. The more dramatic phenotypes seen in $Trl^{R85}$ than in $Trl^{s2325}$and $Trl^{13C\ (40)}$ can be attributed to the fact that $Trl^{R85}$ is a well-established null allele (Farkas et al., 1994). The differences in phenotypes between $Trl^{s2325}$and $Trl^{13C\ (40)}$ are more subtle. Either or both alleles could result in a truncated transcript or in a protein that performs a different role than it normally would. It is likely that because of the location of the $Trl^{s2325}$ P-element insertion that this allele would produce a shorter protein product than $Trl^{13C\ (40)}$. The shorter protein product would likely result in a higher reduction in protein function than in $Trl^{13C\ (40)}$. Previous studies indicate $Trl^{13C\ (40)}$ is a weak hypomorph which supports the results in this study (Greenberg and Schedl, 2001; Bhat et al., 1996). The shorten protein would only contain the BTB/POZ domain which is a protein-protein interaction domain. This means that *Trl* could recruit other proteins to perform its normal function in the ovary. The highest percent of cyst degeneration occurred between stages 2 and 4 in $Trl^{13C\ (40)}$, $Trl^{s2325}$, and $Trl^{R85}$ at about 12, 20, and 85% (respectively) percent of cyst degeneration at those stages of oogenesis out of the total number of cysts counted. When cyst degeneration was looked at in *OtuGal4 > Trl RNAi* ovaries there was significantly more cyst degeneration than the controls and the highest percent of cyst degeneration occurred between stages 7-9 (Figure 4 and 5). Stages 2-4 had already been surpassed when Trl protein is effectively knocked out in *OtuGal4 > Trl RNAi* ovaries resulting in cyst degeneration later in oogenesis instead of in stages 2-4.

The stages where cyst degeneration occurred correspond to key checkpoints in oocyte development. In early oogenesis (stages 2-4), the oocyte goes from being transcriptionally active to transcriptionally quiescent (King, 1970). The oocyte chromatin also becomes tightly compacted by stage 3. These processes ensure that the fate of the oocyte is different from that of the nurse cells. Nutrition-deprived flies undergo programmed cell death (PCD) in mid-oogenesis (stages 7-9) which is thought to be a key checkpoint to ensure optimal use of energy and resources before entering the vitellogenic stages (Pritchett et al., 2009; Peterson et al., 2015). The oocyte also becomes temporarily transcriptionally active again at stage 8 and then immediately reverts to being transcriptionally quiescent in stage 9 (Mahowald and Tiefert, 1970). In stage 9, re-polarization occurs to ensure that the anterior-posterior and dorsal-ventral axes are set up for the embryo (Roth, 2003; Riechmann and Ephrussi, 2001; van Eeden and St Johnston, 1999).

***Trl is necessary for Orb expression in the oocyte***

In this study, I found that *Orb* expression is absent and delayed in *Trl* $^{s2325}$ and *Trl* $^{13C\,(40)}$ respectively (Figure 7). These alleles are both hypomorphs but have different phenotypes. The reason for this could be due to a shorter protein product in *Trl* $^{s2325}$ which would likely result in a higher reduction in protein function than in *Trl*$^{13C(40)}$. The alleles corresponding to FRT80B (*Trl* $^{13C\,(40)}$ and *Trl* $^{s2325}$) were initially favored over the allele corresponding to FRT79D (*Trl*$^{R85}$) because fewer germline only clones were produced using FRT79D. Most of the clones produced using FRT79D were mixed clones (meaning there were both germline and follicle clones in the same cyst). There were very few germline or follicle only clones. Since this study's focus was on the role *Trl* plays in

the germline, a high yield of germline only clones was desirable. FRT80B produced less clones overall but the amount of follicle only, germline only, and mixed clones produced occurred in relatively equal amounts making *Trl* $^{13C(40)}$ and *Trl* $^{s2325}$ initially favorable. It would be beneficial to go back and stain *Trl*$^{R85}$ ovaries with the same antibodies looked at in these studies including *Orb*.

***Trl is not necessary for oocyte chromatin condensation***

Despite the lack of *Orb*, there is still a cell that resembles an oocyte (Figure 11and 12). *Trl* mutant clones had a condensed oocyte nucleus and 15 nurse cells. *Trl* mutants have a different oocyte phenotype than *Orb*, *missing oocyte (Mio)*, and *BicD* mutants which have cysts containing 16 nurse cells with no *Orb* expression (Mach and Lehmann, 1997; Huynh and St Johnston, 2000; Navarro et al., 2004). In *Orb*, *Mio*, and *BicD* mutants, 16 nurse cells with the absence of an oocyte suggest the oocyte is not specified at all. However, *darkener of apricot* (*doa*) mutants, a protein kinase, have a condensed oocyte nucleus and lack *Orb* like the phenotypes seen in the *Trl* mutants in this study (Morris, Navarro, and Lehmann, 2003). This indicates that both *doa* and *Trl* are required for *Orb* accumulation in the oocyte but not oocyte chromatin condensation. *Trl* could be functioning to regulate oocyte-specific transcription of *Orb* in the oocyte before 16-cell cysts.

***Trl is not necessary for oocyte or cyst polarity***

Loss of *Trl* also does not impact *BicD* expression, which like *Orb* is another oocyte-specific factor (Figure 9 and 12). However, *BicD* and *Orb* have different roles in the oocyte and are transcribed and translated at different times. *Orb* is transcribed and

translated by the oocyte along with *Gurken* (*Grk*), another oocyte-specific factor involved in oocyte polarity, in 8-cell cyst and both play a role in oocyte polarity (Figure 12) (Huynh and St Johnston, 2004). *BicD* is transcribed and translated by the nurse cells then is shuttled to the oocyte in 16-cell cysts, and its primary function is in establishing cyst and oocyte polarity (Figure 9 and 12) (Theurkauf et al., 1992; de Cuevas and Spradling, 1998; Huynh and St. Johnston, 2000; Bolivar et al., 2001). Oocyte localization in *Trl* mutants occurred to the posterior pole of the cyst; *BicD* is also more concentrated on the posterior side of the oocyte as is seen in wildtype cysts. In *Trl* mutants, the oocyte is also posteriorly localized within the cyst. Together this suggests that *Trl* is not necessary for cyst or oocyte polarity.

***Trl is not required for oocyte maintenance***

The oocyte not only has to be specified, but the oocyte fate also must be maintained throughout the rest of oogenesis. For instance, *Bazooka* (*Baz*), *PAR-1* and *PAR-6* mutants give rise to cysts that have 16 nurse cells and no oocyte (Huynh et al., 2001a; Mach and Lehmann, 1997; Lantz et al., 1994). *Baz*, *PAR-1*, and *PAR-6* accumulate oocyte-specific proteins in one cell that then never become localized to the posterior of the oocyte (Huynh et al., 2001a; Mach and Lehmann, 1997; Lantz et al., 1994). In these mutants, when the oocyte-specific factors fail to localize to the posterior of the oocyte the cell expressing the oocyte factors exits meiosis and reverts to a nurse cell fate (Huynh et al., 2001a). Thus, *Baz*, *PAR-1*, and *PAR-6* mutants are not required for oocyte specification but are required to maintain the oocyte fate (Huynh et al., 2001b). The otuGal4 driver knocks out gene expression starting in the 16-cell cysts. However, *otuGal4 > Trl RNAi* samples stained with *Trl* antibody had a delay in knocking out *Trl*

expression until stage 2-3 at which time *Trl* is undetectable in the germline. At stage 2, mitotic divisions have been bypassed, and the oocyte is no longer transcriptionally active. The first location of cyst degeneration, stages 2-4, have been bypassed when *Trl* becomes degraded in the RNAi. Oocyte-specific transcripts, *Orb* and *Grk*, and oocyte-specific factors produced by the nurse cells, *BicD* and *Egl*, are already on and in the oocyte of 16-cell cyst. This indicates that *Trl* is not required for oocyte maintenance, which is likely due to the timing of when *Trl* is degraded by the otuGal4 driven RNAi.

Another RNAi driver helped elucidate timing of when *Trl* is needed. The nosGal4-NGT driver knocks out gene expression only slightly in mitotically dividing cyst in Region 2 and 3 of the germarium. *Trl* protein expression is likely not effectively degraded until ~stage 1-2 of oogenesis. The ovarioles do not show any reduction or absence of *Orb,* indicating that oocyte maintenance does not require *Trl*. However, the data does enable us to reconcile that *Trl* is necessary for mitotically dividing cysts.

***Trl is necessary for oocyte-specific transcription of Orb between 8 and late 16-cell cysts***

The data shows that *Trl* is not required for entry into meiosis, oocyte polarity or maintenance, and oocyte chromatin condensation (Figure 12). However, *Trl* is necessary for Orb accumulation in the oocyte. When each of these studies is broken down it narrows down where *Trl* is crucial for Orb accumulation in the oocyte. First, entry into meiosis occurs before 8-cell cyst indicating *Trl* is not required before 8-cell cysts. If you recall, 8-cell cysts are also were there were higher levels of Trl protein. Next, the oocyte chromatin becomes condensed by stage 3 of oogenesis suggesting *Trl* is required before stage 3. Due to Trl protein degradation starting in stage 2, the *Trl RNAi* driven by *OtuGal4* and *NosGal4-NGT* indicated *Trl* is required before stage 2. Lastly, oocyte

polarity occurs in late 16-cell cysts and by stage 1 *BicD* is localized to the posterior pole of the oocyte. This indicates that *Trl* is required before early 16-cell cysts. *Orb* is transcribed and translated by the oocyte between 8-cell cyst and 16-cell cyst. In conclusion this data illustrates that *Trl* is necessary for oocyte-specific transcription in 8 and 16-cell cysts (Figure 12).

The oocyte is transcriptionally active between 8-cell cyst when oocyte specification occurs, and 16-cell cyst and stage 1, when the oocyte is determined and packaged (de Cuevas and Spradling, 1998; Huynh and St. Johnston, 2000). *Orb* and *Gurken*, *Grk,* are transcribed in the oocyte at this time. I hypothesize that *Trl* is required to promote oocyte-specific transcription. The data points to *Trl* having a role after the oocyte is specified, but before the oocyte transcribed oocyte-specific factors. Oocyte-specific transcription could be tested using the 5'ethynyluridine (EU) assay due to its ability for the EU to be incorporated into newly synthesized RNA (Jao and Salic, 2008). I can also use the BamGal4 driver to help elucidate the timing of when *Trl* is necessary for the oocyte. BamGal4 drives transgene expression in mitotically dividing cysts. If *Trl* is required for oocyte-specific transcription in the transition between oocyte specification in 8-cell cyst and oocyte determination and packing in 16-cell cyst then *BamGal4 > Trl RNAi* should lack oocyte-specific transcripts such as *Orb* and *Grk*. However, oocyte-specific factors, such as *BicD* and *Egl*, produced by the nurse cells after mitotic divisions have ceased will be unaffected by *Trl* being knocked out starting in mitotically dividing cysts. These oocyte-specific factors should be unaffected by a loss of *Trl* because *Trl* is not required for their transcription or translation. Thus, *Trl* could be necessary to promote oocyte-specific transcription in 16-cell cysts.

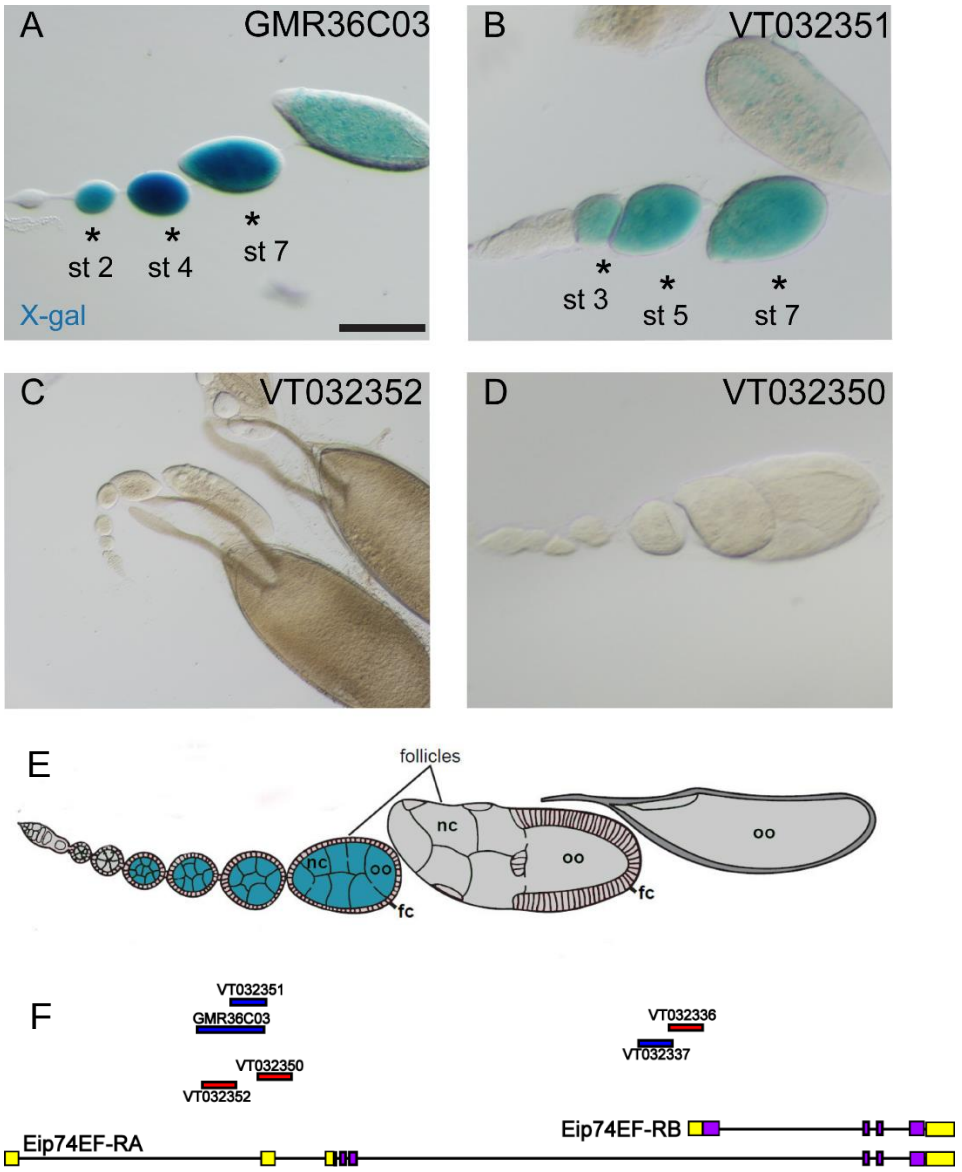


Figure 13: *E74* overlapping enhancer binding site

Fly lines containing small fragments of the *E74* gene locus were screened for transcription factor binding sites. Using the UAS-Gal4 system, when the ovaries were stained with β-galactosidase in the presence of a transcription factor binding site the sample would turn blue. VT032351 and GMR36C03 overlap on the *E74* gene locus (F) and have similar expression patterns. They both show expression in the germline of the ovariole in stages 2 or 3 to stage 7 (A, B, and E). The two fragments that overlap with VT032351 and GMR36C03 do not show positive expression as indicated by the absence of blue when stained with β-galactosidase (C-D). A schematic of the expression pattern is provided (E). Scale bar is 50 μm.

***Trl could be necessary for oocyte-specific transcription of other transcription factors***

*Trl* could also promote oocyte-specific transcription in 16-cell cyst of other factors such as *Ecdysone-induced protein at 74EF* (*E74)*. *E74* is a transcription factor and is required during multiple stages of oogenesis including oocyte development, stem cell function, and early cysts growth and survival (Ables et al., 2012; Buszcak et al., 1999). In a previous study I conducted, fly lines containing regions of the *E74* gene locus were screened, using the UAS-Gal4 system, for enhancer binding sites. The regions identified have very specific expression patterns. GMR36C03 is expressed from stage 2 to stage 8 (Figure 13A). VT032351 is expressed in a similar pattern from stage 3 to stage 7 (Figure 13B). The GMR36C03 and VT032351 fragments overlap with one another and with fragments that were negative (Figure 13F). When the overlapping region was analyzed using MEME-suite, identifies motifs or short recurring DNA sequences that are often binding sites, it showed *Trl* had predicted binding sites within this region. The other enhancer site, VT032337, showed a much larger yet still distinct patterning from 8-cell cyst to stage 10 (Figure 14A). *Trl* also had predicted binding sites within this enhancer region. This data suggests a potential direct interaction between *Trl* and *E74* in these regions. In order to investigate this, the enhancer lines will need to be recombined with Uasp-lacZ and once established, crossed with the *Trl RNAi*. Using this recombined line with the *Trl RNAi,* if *Trl* promotes *E74* at these enhancer sites, then a loss of *Trl* should resemble *E74* loss-of-function mutants. *E74* loss-of-function mutants fail to develop past early oogenesis (stages 2-4) (Buszczak et al., 1999). The premature death in *E74* mutants in early oogenesis is similar to the cyst degeneration seen in *Trl* mutants in stages 2-4.

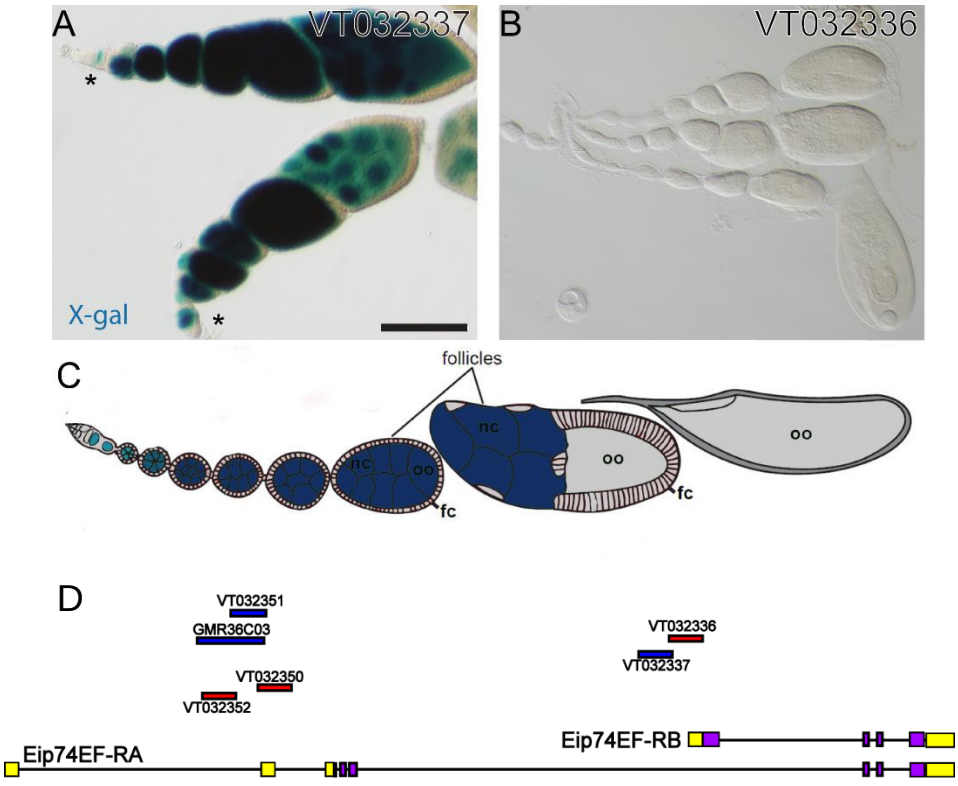


Figure 14: *E74* enhancer binding site

Fly lines containing small fragments of the *E74* gene locus were screened for transcription factor binding sites. Using the UAS-Gal4 system, when the ovaries were stained with β-galactosidase in the presence of a transcription factor binding site the sample would turn blue. VT032337 has a similar yet distinct expression pattern to that seen in VT032351 and GMR36C03. VT032337 has expression in the germline of the ovariole in 8-cell cyst to stage 10 of oogenesis (A and C). The fragment that overlaps with VT032337 does not show positive expression as indicated by the absence of blue when stained with β-galactosidase (B). A schematic of the expression pattern is provided (E). Scale bar is 100 μm.

There is still more research that needs to be done to understand the role *Trl* plays in promoting oocyte-specific transcription of oocyte-specific factors before the oocyte becoming quiescent. Understanding the role *Trl* plays in the oocyte can help us understand how chromatin modification affects the critical set of conserved, developmental steps a mature oocyte must undergo. In turn, it could also help us understand these developmental steps in other species.

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