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Analysis of Cell Cycle Switches in *Drosophila* Oogenesis

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Abstract

The study of *Drosophila* oogenesis provides invaluable information about signaling pathway regulation and cell cycle programming. During *Drosophila* oogenesis, a string of egg chambers in each ovariole progressively develops toward maturity. Egg chamber development consists of 14 stages. From stage 1 to stage 6 (mitotic cycle), main-body follicle cells undergo mitotic divisions. From stage 7 to stage 10a (endocycle), follicle cells cease mitosis but continue three rounds of endoreduplication. From stage 10b to stage 13 (gene amplification), instead of whole genome duplication, follicle cells selectively amplify specific genomic regions, mostly for chorion production. So far, *Drosophila* oogenesis is one of the most well studied model systems used to understand cell cycle switches, which furthers our knowledge about cell cycle control machinery and sheds new light on potential cancer treatments. Here, we give a brief summary of cell cycle switches, the associated signaling pathways and factors, and the detailed experimental procedures used to study the cell cycle switches.

Keywords

Endocycle; Mitotic cycle; Gene amplification; *Drosophila* oogenesis; Follicle cell

1 Introduction

In *Drosophila*, the female has two ovaries, each of which is composed of 16–20 ovarioles. The ovariole is a string of progressively developing egg chambers. Each egg chamber, the

It is important to feed flies with yeast for 2–3 consecutive days before dissection in order to fatten the ovaries prior to harvesting.

It is necessary to break the muscular sheath and tease apart the ovarioles, in order to obtain better antibody staining results.

For antibody staining, as long as the primary antibodies are not from the same species, they can be mixed and co-stained with corresponding secondary antibodies using in different fluorescence channels. However, in our experience, when BrdU incorporation is done it cannot co-stain with other antibodies. Although an EdU incorporation assay allows for co-staining, its quality is not as good as BrdU incorporation in follicle cells.

Some primary antibodies are very expensive or difficult to obtain. In this case, we suggest to reuse the antibodies. Exactly how many times they can be reused depends on the quality of the antibodies.

Sometimes, the quality of antibodies varies between lots/batches, including commercially available ones.

Since the mounting solution is very sticky, it is hard to transfer exactly 120 μ l into a tube. As an easy alternative, use a 1000 μ l pipette tip to transfer three drops of mounting solution into the tube.

The thickness of the cover glass depends on the confocal imaging system.

Sealing the edges of the cover glass is necessary to avoid the egg chambers from flowing inside of the mounting solution when taken for confocal imaging. The nail polish mentioned in **step 26**, Subheading 3.1 should be transparent.

From the FACS analysis, we can determine the 2C, 4C, 8C, 16C, and 32C peaks. Based on the results, follicle cells that undergo extra or fewer cell cycles can be determined.

Most importantly, keep in mind which cell cycle program is being tested and apply the corresponding primary antibodies to study the cell cycle switches.

developmental unit of oogenesis, consists of 16 germ-line cells, one oocyte and 15 nurse cells, covered by a single layer of somatic follicle cells [1]. Egg chamber development is divided into 14 stages. Based on the cell cycle programs of the main-body follicle cells, egg chamber stages are further grouped into three different categories: the mitotic cycle (stages 1–6), the endocycle (stages 7–10a), and gene amplification (stages 10b–13). A stage-14 egg chamber is the mature egg, ready to be laid. During the mitotic cycle, the main-body follicle cells undergo mitotic divisions for a total of 8–9 rounds with complete cell cycles (G1, S, G2, M phases), increasing the follicle cell number to approximately 650 [2, 3]. During the endocycle, follicle cells continue an additional three rounds of endoreduplication without dividing, skipping the G2 and M phases. During gene amplification, the follicle cells cease whole genome duplication, and instead selectively amplify specific genomic regions, mostly related to chorion (egg shell) production [4 – 7].

For more than a decade, researchers have intensively studied oogenesis to understand how *Drosophila* precisely control mitotic cycle/endocycle (M/E) and endocycle/gene amplification (E/A) switches during oogenesis. Many publications, including ours, have provided evidence that the Notch pathway is a key player in regulating the switches of the cell cycle programs in egg chamber development, which signals between the germ line and follicle cells to coordinate their development [8, 9]. Notch signaling activation at stages 6/7 transitions the mitotic cycle into the endocycle and its inactivation at stages 10a/10b switches the endocycle into gene amplification [4, 10 – 12]. In addition to Notch signaling, other pathways also have been found involved in cell cycle regulation. The Hippo pathway promotes Notch signaling during the M/E switch [13]. Shaggy and Dishevelled, downstream factors of the Wntless pathway, independently potentiate Notch signaling for the M/E switch [14, 15]. Ecdysone signaling activity at stage 10b is required in main-body follicle cells for proper entry into gene amplification [12].

Here, we discuss the methods used to analyze cell cycle switches using available markers and reporters. Follicle cells undergo rounds of complete cell cycles (G1, S, G2, M phases) in mitotic cycle, but skip G2 and M phases during the endocycle and gene amplification. Antibodies against mitotic cell cycle markers, including Cyclin A (CycA) for labeling G2 and M phases, Cyclin B (CycB) and Phospho-Histone-3 (PH3) for M phase, can be applied in follicle cells to specifically mark the mitotic cycle. Otherwise, absence of these mitotic markers suggests follicle cells have ceased mitosis. For S phase detection, the presence of Cyclin E (CycE), MPM2 (Antiphospho-Ser/Thr-Pro), dE2F1, Origin recognition complex (ORC) and incorporation of Bromodeoxyuridine (5-bromo-2'-deoxyuridine, BrdU) is crucial. CycE together with Cyclin-dependent kinase 2 (cdk2) is required for the G1/S transition. The MPM2 antibody has been developed to recognize the CycE/Cdk2-dependent phosphoepitope at the histone locus body [16]. The ORC is required for gene replication during S phase, and dE2F1 restricts the ORC2 to amplification foci [12, 17]. BrdU is an analog of thymidine, which has been applied to the detection of newly synthesized DNA. CycE, dE2F1, MPM2-staining, ORC2 and BrdU-incorporation oscillate during the mitotic cycle and the endocycle, which changes to uniform expression during gene amplification as main-body follicle cells synchronously allow specific genomic regions to be replicated. Antibodies against CycE and dE2F1 present donut-like staining at stage 10b, while MPM2, ORC2, and BrdU staining show a unique foci-like pattern [5, 12, 17]. Failure to detect a

uniform CycE, dE2F1, MPM2, ORC2, and BrdU pattern at stage 10b would be indicative of a defective E/A switch. Newly developed FLY-FUCCI lines (available from the Bloomington *Drosophila* stock center) have worked successfully in imaginal discs and some other tissues to distinguish G1, S, G2 phases [18]. Unfortunately, ubi-mRFP-CycB, a key component of the FLY-FUCCI system, is not detectable in the egg chamber system (our lab unpublished data). However, FLY-FUCCI UAS lines still represent a possible way to identify the cell cycle phase of each follicle cell. Besides cell cycle markers, some cell fate markers may also be used to indicate cell cycles. In early oogenesis, main-body follicle cells are not fully differentiated and can be labeled with Fasciclin III (FasIII) and Eye absent (Eya), which are considered immature cell fate markers. Later, in endocycling (“mature”) main-body follicle cells, FasIII is no longer detectable and Eya expression is severely reduced between stages 7 and 8 before becoming completely absent after stage 8 [5, 6, 19]. Thus, main-body follicle cells labeled by FasIII and Eya maintain an immature cell fate, suggesting they are in the mitotic cycle program. Moreover, some signaling pathway reporters and transcription factors can identify the cell cycle program in follicle cells as well, such as the Notch signaling reporters *E(spl)mβ-CD2* [20], *E(spl)m7-lacZ* [21], *Gbe+Su(H)m8-lacZ* [22], and *NRE-EGFP* [23] and its downstream factors Cut, Hindsight (Hnt), and Broad (Br) [5, 6, 19]. Hnt, Br, and Notch signaling reporters are upregulated by Notch signaling during the M/E switch, while Cut, a Notch negative target during oogenesis, is suppressed. Br has been confirmed to be directly regulated by Notch signaling via the *brE* enhancer [19]. Defective Notch activity has been shown to disrupt the M/E and E/A switches, and corresponding cell cycle programs as well. There is also a Zinc-finger protein, Tramtrack (Ttk), which must be dramatically upregulated at stage 10b for proper gene amplification entry [12]. All markers and reporters mentioned above are summarized in Fig. 1. Additionally, ploidy of DAPI-stained follicle cell nuclei can be estimated based on nuclear volume, and applying fluorescence-activated cell sorting (FACS) analysis can select specific groups of cells to further determine DNA copy number and thus decipher their endocycle progression. Mitotic cycling follicle cells are diploid, showing a 2C peak by FACS analysis. Endocycling follicle cells undergo three rounds of endoreduplication, demonstrating distinctive 4C, 8C, and 16C peaks. Follicle cells that undergo an extra cell cycle can be determined by the appearance of a 32C peak (see [12]). Follicle cells with defective endocycle progression show a reduced 16C peak. Detailed experimental procedures are illustrated below.

2 Materials

2.1 Fly Stocks

Notch activity reporters: *E(spl)mβ-CD2* [20], *E(spl)m7-lacZ* [21], *Gbe + Su(H)m8-lacZ* [22], *NRE-EGFP* (#30727, #30728; Bloomington *Drosophila* Stock Center) [23], *brE-GFP* and *brE-lacZ* [19, 24].

2.2 Antibodies

1. Primary antibodies :Rabbit anti-CycA 1:500 (provided by C. Lehner, University of Zurich, Zurich, Switzerland), mouse anti-CycB 1:5 (F2F4; Developmental Studies Hybridoma Bank, DSHB), guinea pig anti-CycE 1:500 (provided by T. Orr-Weaver, Whitehead Institute, Cambridge, MA), rabbit anti-PH3 1:200

(Ser10, Millipore), mouse anti-BrdU 1:50 (BD Biosciences), mouse anti-FasIII 1:15 (7G10; DSHB), mouse anti-Eya 1:10 (10H6; DSHB), mouse anti-CD2 1:50 (AbD Serotec), rabbit anti- β -galactosidase 1:2000 (MP Biomedicals), mouse anti- β -galactosidase 1:500 (Promega), rabbit anti-Cut 1:500 (provided by Y.N. Jan, University of California, San Francisco, CA), mouse anti-Cut 1:15 (2B10; DSHB), anti-Hnt 1:15 (1G9; DSHB), mouse anti-Br-core 1:30 (25E9; DSHB), rabbit anti-Ttk69 and rat anti-Ttk69 1:200 (provided by P. Badenhorst, University of Birmingham, Edgbaston, Birmingham, UK), guinea pig anti-dE2F1 1:500 (provided by T. Orr-Weaver, Whitehead Institute, Cambridge, MA), rabbit anti-ORC2 1:3000 (provided by S.P. Bell, Whitehead Institute, Cambridge, MA), mouse anti-Ser/Thr-ProMPM-2 1:1000 (Millipore).

2. Secondary antibodies: According to primary antibodies, select corresponding Alexa Fluor 488, 546, or 633 secondary antibodies at a dilution of 1:400.

2.3 Reagents

1. 1× PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄.
2. 1× PBT: 1× PBS supplemented with 0.2 % Triton™ X-100.
3. 1× PBT (0.4 % T): 1× PBS supplemented with 0.4 % Triton™ X-100.
4. Grace's insect medium.
5. Fix solution: 4 % formaldehyde (EM Grade) in 1× PBS.
6. PBTG: 0.2 % bovine serum albumin, 5 % normal goat serum in 1× PBT.
7. Mounting solution: 1 g *n*-propyl gallate, 5 ml of 10× PBS, 40 ml of glycerol and 5 ml of dH₂O.
8. Buffer B: 100 mM KH₂PO₄/K₂HPO₄, pH 6.8, 450 mM KCl, 150 mM NaCl, 20 mM MgCl₂.
9. DNase I.
10. DNase I reaction buffer: 3.3 ml of 1 M Tris-HCl, pH 7.5, 0.125 ml of 2 M MgCl₂, 0.004 ml of 2-mercaptoethanol (98 %) and 46.575 ml of dH₂O.
11. 10 % fetal calf serum.
12. BrdU.
13. DAPI (10 µg/ml) for DNA/nuclei staining.
14. 0.25 % trypsin (diluted in 1× PBS).

2.4 Equipment

1. Dissecting forceps.
2. Embryo dish.
3. CO₂ tank and pad.

4. Centrifuge for eppendorf tubes.
5. Nutator.
6. Stereoscopic (dissecting) microscope.
7. Confocal microscope.
8. Flow cytometer.

3 Methods

3.1 Classic Immunostaining of Ovaries [25]

1. Feed the flies with yeast for 2–3 consecutive days before dissection (*see* Note 1).
2. Fill the embryo dish with 3 ml of 1× PBS.
3. Anesthetize the flies on a CO₂ pad.
4. Select a female fly, then use a pair of dissecting forceps to gently grab the lower thorax and submerge it into a 1× PBS solution in the embryo dish.
5. Pinch the lower abdomen with another pair of forceps, then pull gently to expose the internal organs.
6. Find the pair of ovaries and detach them from the fly body.
7. At the posterior end of the ovaries, break the muscular sheath and tease apart the ovarioles (*see* Note 2).
8. Transfer the ovaries into 500 µl of 1× PBS into a 1.5 ml eppendorf tube, while placing the tube on ice until enough ovaries are collected.
9. Remove the 1× PBS and add 0.5 ml of fix solution.
10. Place the tube to rock on a nutator for 10 min.
11. Remove the fix solution and discard in an appropriate waste container.
12. Wash the ovaries 3 × 15 min in 1 ml of 1× PBT.
13. Remove last PBT wash and add 1 ml of PBTG to block nonspecific binding. Rock on a nutator for 1 h at room temperature or overnight at 4 °C.
14. Remove the PBTG, add 150 µl of primary antibody (select desired markers to test for cell cycle switches). Incubate overnight at 4 °C with rocking (*see* Notes 3–5).
15. Remove the primary antibody (return to a 1.5 ml eppendorf tube for reuse) and wash 3 × 15 min with 1 ml of 1× PBT.
16. Add 150 µl of secondary antibody, incubate for 2 h at room temperature or overnight at 4 °C with rocking.
17. Remove the secondary antibody (return to a 1.5 ml eppendorf tube for reuse) and wash 2 × 15 min with 1 ml of 1× PBT.

18. Add 150 μ l of DAPI (10 μ g/ml) for 10–15 min.
19. Remove the DAPI and wash 1×10 min with 1 ml of $1 \times$ PBT.
20. Discard the PBT and wash 2×10 min with 1 ml of $1 \times$ PBS.
21. Remove the PBS leaving about 300 μ l in the tube with the ovaries.
22. Using a 200 μ l pipette tip, pipet the ovaries up and down several times to free the egg chambers.
23. Spin down slightly and remove as much $1 \times$ PBS as possible.
24. Add 120 μ l of mounting solution (*see* Note 6).
25. Cut approximately 0.3 mm off the tip of a 200 μ l pipette tip, then use it to transfer the mounting solution with the egg chambers to a microscope glass slide.
26. Cover the solution with a coverslip glass and seal the edges with nail polish (*see* Notes 7 and 8).

3.2 BrdU Incorporation Assay

1. For dissection the ovaries follow the **steps 1–8** in Subheading 3.1, except replace the $1 \times$ PBS solution with Grace's insect medium.
2. Remove the ovaries from Grace's insect medium into 1 ml of a BrdU solution (0.5 mg/ml BrdU in Grace's insect medium). Incubate for 1 h at room temperature.
3. Remove the BrdU solution and discard in an appropriate waste container. Wash 2×3 min with 1 ml of $1 \times$ PBS.
4. Remove and discard the $1 \times$ PBS. Fix the ovaries for 15 min in 500 μ l of fix solution (32 % EM grade formaldehyde, Buffer B, and H_2O at 1:1:4).
5. Remove the fix solution and discard in an appropriate waste container. Wash 2×15 min with 1 ml of $1 \times$ PBT.
6. Remove and discard the PBT. Wash the ovaries 2×15 min with 1 ml of DNase I reaction buffer.
7. Remove the DNase I reaction buffer and discard in sink. Add DNase I (0.1 U/ μ l) in 1 ml of DNase I reaction buffer. Incubate for at least 45 min at 37 °C. Remove and discard the DNase I and the buffer.
8. Wash 3×1 min with 1 ml of $1 \times$ PBT.
9. Wash 2×15 min with 1 ml of $1 \times$ PBT.
10. For anti-BrdU antibody labeling, DAPI staining, and preparing the ovaries for mounting, follow the **steps 13–26** as described in Subheading 3.1.

3.3 Fluorescence-Activated Cell Sorting (FACS) Analysis [12, 26]

1. Follow the steps in Subheading 3.1 **step 1–8**, except replace the 1× PBS solution with Grace's insect medium, supplemented with 10 % fetal calf serum and 1× antibiotic–antimycotic. Approximately 80–150 females per experiment are needed for dissection.
2. Wash the ovaries 3 × 2 min in 1 ml of 1× PBS.
3. Incubate the ovaries for 15 min in 0.7 ml of 0.25 % trypsin (diluted in 1× PBS) with intermittent vortexing at room temperature.
4. To isolate the follicle cells, remove the supernatant and pass it through a 40-µm nylon filter into 1 ml of Grace's insect medium.
5. Centrifuge at 1700 × *g* (4000 rpm) for 7 min.
6. Repeat the trypsinization and filtration steps 2–3× or until the supernatant becomes clear.
7. Resuspend the follicle cells in 0.5 ml of Grace's insect medium containing 1 µl of 5 mM Vybrant DyeCycle DNA-specific stain, and incubate for 30 min at room temperature.
8. Rinse 1× with 1 ml of 1× PBS and store on ice.
9. Use a flow cytometer to determine the follicle cell ploidy by FACS analysis of Vybrant DyeCycle-stained cell preparations with excitation at 407 nm for Vybrant DyeCycle stain and at 488 nm for GFP (*see* Notes 9 and 10).

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References

1. Spradling, A. Developmental genetics of oogenesis The development of *Drosophila melanogaster*. Cold Spring Harbor Lab Press; New York: 1993.
2. Nystul T, Spradling A. Regulation of epithelial stem cell replacement and follicle formation in the *Drosophila* ovary. *Genetics*. 2010; 184:503–515. [PubMed: 19948890]
3. Skora AD, Spradling AC. Epigenetic stability increases extensively during *Drosophila* follicle stem cell differentiation. *Proc Natl Acad Sci U S A*. 2010; 107:7389–7394. [PubMed: 20368445]
4. Calvi BR, Lilly MA, Spradling AC. Cell cycle control of chorion gene amplification. *Genes Dev*. 1998; 12:734–744. [PubMed: 9499407]
5. Sun J, Deng WM. Notch-dependent downregulation of the homeodomain gene cut is required for the mitotic cycle/endocycle switch and cell differentiation in *Drosophila* follicle cells. *Development*. 2005; 132:4299–4308. [PubMed: 16141223]
6. Sun J, Deng WM. Hindsight mediates the role of notch in suppressing hedgehog signaling and cell proliferation. *Dev Cell*. 2007; 12:431–442. [PubMed: 17336908]
7. Shyu LF, Sun J, Chung HM, et al. Notch signaling and developmental cell-cycle arrest in *Drosophila* polar follicle cells. *Mol Biol Cell*. 2009; 20:5064–5073. [PubMed: 19846665]

8. Ruohola H, Bremer KA, Baker D, et al. Role of neurogenic genes in establishment of follicle cell fate and oocyte polarity during oogenesis in *Drosophila*. *Cell*. 1991; 66:433–449. [PubMed: 1907889]
9. Xu T, Caron LA, Fehon RG, et al. The involvement of the Notch locus in *Drosophila* oogenesis. *Development*. 1992; 115:913–922. [PubMed: 1451667]
10. Deng WM, Althausen C, Ruohola-Baker H. Notch-Delta signaling induces a transition from mitotic cell cycle to endocycle in *Drosophila* follicle cells. *Development*. 2001; 128:4737–4746. [PubMed: 11731454]
11. Lopez-Schier H, St Johnston D. Delta signaling from the germ line controls the proliferation and differentiation of the somatic follicle cells during *Drosophila* oogenesis. *Genes Dev*. 2001; 15:1393–1405. [PubMed: 11390359]
12. Sun J, Smith L, Armento A, et al. Regulation of the endocycle/gene amplification switch by Notch and ecdysone signaling. *J Cell Biol*. 2008; 182:885–896. [PubMed: 18779369]
13. Yu J, Poulton J, Huang YC, et al. The hippo pathway promotes Notch signaling in regulation of cell differentiation, proliferation, and oocyte polarity. *PLoS One*. 2008; 3:e1761. [PubMed: 18335037]
14. Axelrod JD, Matsuno K, Artavanis-Tsakonas S, et al. Interaction between Wingless and Notch signaling pathways mediated by dishevelled. *Science*. 1996; 271:1826–1832. [PubMed: 8596950]
15. Jordan KC, Schaeffer V, Fischer KA, et al. Notch signaling through tramtrack bypasses the mitosis promoting activity of the JNK pathway in the mitotic-to-endocycle transition of *Drosophila* follicle cells. *BMC Dev Biol*. 2006; 6:16. [PubMed: 16542414]
16. White AE, Leslie ME, Calvi BR, et al. Developmental and cell cycle regulation of the *Drosophila* histone locus body. *Mol Biol Cell*. 2007; 18:2491–2502. [PubMed: 17442888]
17. Royzman I, Austin RJ, Bosco G, et al. ORC localization in *Drosophila* follicle cells and the effects of mutations in dE2F and dDP. *Genes Dev*. 1999; 13:827–840. [PubMed: 10197983]
18. Zielke N, Korzeliuss J, van Straaten M, et al. Fly-FUCCI: A versatile tool for studying cell proliferation in complex tissues. *Cell Rep*. 2014; 7:588–598. [PubMed: 24726363]
19. Jia D, Tamori Y, Pyrowolakis G, et al. Regulation of broad by the Notch pathway affects timing of follicle cell development. *Dev Biol*. 2014; 392:52–61. [PubMed: 24815210]
20. de Celis JF, Tyler DM, de Celis J, et al. Notch signalling mediates segmentation of the *Drosophila* leg. *Development*. 1998; 125:4617–4626. [PubMed: 9806911]
21. Assa-Kunik E, Torres IL, Schejter ED, et al. *Drosophila* follicle cells are patterned by multiple levels of Notch signaling and antagonism between the Notch and JAK/STAT pathways. *Development*. 2007; 134:1161–1169. [PubMed: 17332535]
22. Furriols M, Bray S. A model Notch response element detects Suppressor of Hairless-dependent molecular switch. *Curr Biol*. 2001; 11:60–64. [PubMed: 11166182]
23. Saj A, Arziman Z, Stempfle D, et al. A combined ex vivo and in vivo RNAi screen for notch regulators in *Drosophila* reveals an extensive notch interaction network. *Dev Cell*. 2010; 18:862–876. [PubMed: 20493818]
24. Fuchs A, Cheung LS, Charbonnier E, et al. Transcriptional interpretation of the EGF receptor signaling gradient. *Proc Natl Acad Sci U S A*. 2012; 109:1572–1577. [PubMed: 22307613]
25. Wong LC, Schedl P. Dissection of *Drosophila* ovaries. *J Vis Exp*. 2006; 52
26. Bryant Z, Subrahmanyam L, Tworoger M, et al. Characterization of differentially expressed genes in purified *Drosophila* follicle cells: toward a general strategy for cell type-specific developmental analysis. *Proc Natl Acad Sci U S A*. 1999; 96:5559–5564. [PubMed: 10318923]

Fig. 1. The cell cycle switches during *Drosophila* oogenesis

Follicle cells undergo a mitotic cycle (stages 1–6, complete G1, S, G2, M phases), an endocycle (stages 7–10a, G1, S phases), and gene amplification (stages 10b–13, DNA synthesis at specific genomic loci). Specific cell cycle markers (CycA, CycB, PH3, CycE, dE2F1, MPM2, ORC2, BrdU), cell fate markers (FasIII, Eya), and signaling reporters and factors (*E(spl)mβ-CD2*, *E(spl)m7-lacZ*, *Gbe+Su(H)m8-lacZ*, *NRE-EGFP*, Hnt, Br, *brE*, Cut, Ttk) can be applied to determine the follicle cell cycle programs