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At the crossroads of differentiation and proliferation: Precise control of cell-cycle changes by multiple signaling pathways in *Drosophila* follicle cells

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Abstract

Here, we discuss the findings to date about genes and pathways required for regulation of somatic follicle-cell proliferation and differentiation during *Drosophila* oogenesis and demonstrate how loss of these genes contributes to the tumorigenic potential of mutant cells. Follicle cells undergo cell-fate determination through stepwise activation of multiple signaling pathways, including the Notch, Hedgehog, Wingless, janus kinase/STAT, and JNK pathways. In addition, changes in DNA replication and cellular growth depend on the spatial and temporal activation of the mitotic cycle-endocycle and endocycle-gene amplification cell-cycle switches and insulin-dependent monitoring of cellular health; systemic loss of these pathways contributes to loss of controlled cellular proliferation, loss of differentiation/growth, and aberrant cell polarity in follicle cells. We also highlight the effects of the neoplastic and Hippo pathways on the cell cycle and cellular proliferation in promoting normal development and conclude that lack of coordination of multiple signaling pathways promotes conditions favorable for tumorigenesis.

Keywords

cell cycle; cell differentiation; follicular epithelium; oogenesis; tumorigenesis

Introduction

A pervasive threat to human health and longevity is cancer, the overall result of uncontrolled cellular proliferation that can metastasize, invade neighboring tissues, and impede the function of major organs to the point of death. Cancer affects many cellular systems to achieve tumorous growth, such as the cell cycle, checkpoints [1], and cellular differentiation [2]. Recent research has focused on therapeutic targets of several signaling pathways activated in various human cancers, including Notch [3], Hedgehog (Hh), and janus kinase (JAK/STAT) [4], in the hope of deterring proliferation and cancer progression. Given that multiple pathways are affected in a single cancerous cell, determining the hierarchy of gene expression and pathway activation that promotes tumorigenesis is difficult. *Drosophila* oogenesis, however, provides a versatile model with which to study stage-specific activation of signaling pathways that regulate cell-fate determination, cellular proliferation, and growth. Careful analysis of this model can provide insights into the conditions that promote tumorigenesis and therefore improve cancer therapies and prognosis of survival.

Each female *Drosophila melanogaster* has two ovaries, each with 16–20 ovarioles (muscular sheaths containing egg chambers that progressively mature in preparation for

fertilization and embryogenesis; Fig. 1). At the anterior end of each ovariole is the germarium (Fig. 2A), which houses stem cells for germ-line and somatic lineages, the anterior tip of which consists of specialized cells (cap cells, escort cells, and terminal filament) that maintain microenvironments called niches [5] essential for germ-line stem cell proliferation and maintenance [6]. Each stem cell divides to produce a daughter cell and a cystoblast. The cystoblast undergoes four mitotic divisions with incomplete cytokinesis to form a 16-cell syncytium, in which one cell becomes the oocyte and the other 15 become nurse cells. This syncytium is enveloped by a single layer of somatic follicle cells derived from the follicle stem cells (FSCs) to create a newly formed egg chamber [7]. The egg chamber buds off from the germarium and pursues development through 14 distinct stages; only the oocyte survives during the transition to embryogenesis [8–10].

The follicular epithelium contributes significantly to egg-chamber maturation and survival, including establishment and maintenance of oocyte polarity, nutrient uptake [11], eggshell production [11], and multiple egg-chamber homeostatic pathways that monitor the health of the egg chamber and modulate growth and arrest in response to environmental cues [12, 13]. During oogenesis, follicle cells exhibit three different cell-cycle regimens and stepwise differentiation of distinct cell subpopulations through multiple signaling pathways (Fig. 3A–C) [14]. Follicle cells first undergo mitosis from the germarium to stage 6 (termed early oogenesis), marked by expression of mitotic genes and reporters [15]. Disappearance of these markers is correlated with the onset of the mitotic cycle–endocycle (M/E) switch [16,17], which allows oscillating Cyclin E (CycE) expression to drive the endocycle, a variant of the cell cycle in which mitosis is skipped while genomic DNA still replicates [18]. The endocycle phase (termed middle or midoogenesis) lasts until the endocycle–gene amplification (E/A) switch at stage 10B. During gene-amplification stages (stage 10B to late, termed late oogenesis), specific genomic loci (e.g. chorion genes) are selectively replicated to produce enormous amounts of eggshell protein, needed to protect the mature oocyte/embryo from the outside environment. The gene-amplification cycles are marked by uniform expression of CycE and characteristic bromodeoxyuridine (BrdU; dU analog) incorporation foci at the chorion gene loci [19, 20]. This review will present the genetic data published to date on pathways and genes involved in cell-cycle control, cellular proliferation and differentiation, and their roles in promotion of the tumorigenic potential of aberrant cells in the somatic epithelium of *Drosophila* oogenesis (Table 1).

Early oogenesis: The mitotic stages

During early oogenesis, follicle cells undergo 8–9 rounds of archetypal G2-M (mitosis) and G1-S (replication), which increase their number to roughly 650 so as to maintain a contiguous epithelium as the egg chamber grows [21, 22]. Mitosis is readily detected in follicle-cell precursors generated from the FSCs by the oscillating expression of mitotic-cycle markers [15, 21, 23]. During these stages, multiple signaling pathways promote follicle-cell mitosis and/or polarization of the egg chamber.

In the canonical Wingless (Wg) pathway, Wg initiates a signaling cascade that results in binding of Pangolin (Png; *Drosophila* homolog of TCF) by Armadillo (Arm; *Drosophila* homolog of β -catenin) and activation of transcription of downstream targets [24]. During oogenesis, Wg expression is limited to the terminal filament and cap cells [25]; loss of the Polycomb group genes *suppressor of zeste 2* (*Su(z)2*) and *posterior sex combs* (*psc*) shows upregulation of Wg expression in mutant follicle cells, implying that Polycomb repression complexes downregulate Wg expression in follicle cells [26].

Intriguingly, certain downstream components of the Wg pathway such as *shaggy* (*sgg*) and *axin*, when mutated, produce defects in the M/E switch [25, 27]; others, such as *arm* and

pygopus (*pygo*; canonical Wg transcription factor component), do not [27]. Whether loss of the Wg scaffolding protein *dishevelled* (*dsh*) affects follicle cells is unclear, as previous studies of the *dsh*^{VA135} and *dsh*⁴⁷⁷ alleles reveal discrepancies in the rates of mitotic division [25, 27]. Reduction and overexpression of Wg signaling in the germarium both affect FSC maintenance [25]; because Wg expression in FSCs appears to be detrimental, more research must be conducted to determine whether the phenotypes manifested by *sgg*, *axin*, and *dsh* loss of function (LOF) clones depend on their expression in FSCs in conjunction with Wg signaling in the niche.

Hh signaling, like the Wg pathway, is also limited to the terminal filament and cap cells of the germarium, and loss or gain of Hh signaling in the germarium also affects FSC maintenance and division [23, 28]. The Hh pathway components *patched* (*ptc*), *protein kinase a* (*pka*), and the nuclear effector *cubitus interruptus* (*ci*) are expressed from the germarium to stage 6 in oogenesis before being downregulated [29]. Because Ptc, the Hh receptor, suppresses the activity of the transmembrane protein Smoothened [30, 31], loss of *ptc* results in excessive Ci expression and proliferation of mitotic tissue [23, 29, 32]. PKA phosphorylates the large isoform of Ci (Ci-155) for cleavage into the smaller Ci-75 isoform, marking it for eventual degradation [33, 34]; loss of *pka* results in defective degradation and higher levels of Ci than in wild-type siblings during early oogenesis [29]. Unlike Wg, the major components of Hh signaling studied to date are required for oogenesis, but it still relies on faithful niche communication, illustrating the importance of stem-cell niches in controlling spatiotemporal regulation of multiple signaling pathways.

The c-Jun N-terminal kinase (JNK) pathway is also active throughout the mitotic stages of *Drosophila* oogenesis. A role for JNK signaling in mitosis was uncovered through LOF studies in JNK pathway genes *basket* (*bsk*), *hemipterous* (*hep*), and *puckered* (*puc*) [27]. Inactivation of these genes can either activate the endocycle early (*bsk*, *hep*) or arrest the cell in a mitotic state (*puc*) [27]. Whether the role of JNK signaling in mitosis is conserved in different tissues and species is not yet known; recent research has implicated JNK signaling in phosphorylation of Cdc25C (human homolog of Stg) in human-cell cultures [35], as well as promotion of Aurora B kinase expression and subsequent histone H3 phosphorylation [36]. Therefore, JNK signaling may still be required for phosphorylation of substrates needed for proper mitotic activity.

JAK/STAT is one pathway required for proper egg development during oogenesis. Specialized “polar” cells (located at the termini of the egg chamber) express the JAK/STAT ligand Unpaired (Upd) at high levels. The initiation of the JAK/STAT pathway in polar cells (PCs) by Upd (and maintenance of JAK/STAT signaling by the GTPase RalA) produces morphogen gradients at both anterior and posterior ends of the egg chamber and is thought to maintain the chamber’s anteroposterior axis, in addition to activating downstream targets of the pathway [37, 38]. Loss of JAK/STAT activation/maintenance leads to cellular-differentiation defects throughout oogenesis and loss of egg polarity [39,40].

Several other genes important for mitosis in main-body follicle cells (MbFCs) also prevent aberrant cell-cycle changes in mitotically dividing follicle cells. For example, *cut* encodes a CCAAT-binding and homeodomain transcription factor whose mammalian homolog Cux1 is known to activate and repress transcription of targets in a context-dependent manner [41]. The pathway that activates Cut expression in the germarium is unknown; nevertheless Cut expression is required in MbFCs during early oogenesis for mitosis to occur, as loss of *cut* before stage 6 suffices to terminate mitosis and prematurely activate the endocycle [42]. Another example is *extramacrochaete* (*emc*), which encodes a bHLH protein that lacks a DNA-binding domain and cannot activate transcription of genes. Instead, Emc antagonizes the binding of other bHLH proteins to transcriptional targets [43]. FSC clones of an *emc*

mutation show reduced expression of Eyes Absent (Eya) [44], a tyrosine phosphatase expressed ubiquitously in mitosis except in PCs [45]. *eya*'s primary role is to prevent mitotic follicle cells from differentiating into PCs, which are in cell-cycle arrest [45]; *emc* is therefore needed to promote Eya expression during mitosis.

In summary, components of the JNK, Wg, and Hh signaling pathways are required for proper mitotic progression during early oogenesis, although whether downstream components are activated directly by Wg and Hh is unclear. In addition to those promoting mitosis, some genes actively suppress cell-cycle deviations in follicle cells during early oogenesis. Finally, JAK/STAT signaling provides one of the initial egg-chamber polarization cues that will facilitate cellular differentiation during transition to midoogenesis.

Transition to midoogenesis: The endocycle stages

During midoogenesis (stages 7–10A; Fig. 4), the follicle cells focus exclusively on Notch-dependent cellular growth by switching to the endocycle, of which they undergo three rounds within 24 hours at 25°C [8, 46, 47]. Notch is a trans-membrane receptor activated by the germ-line-derived Dl ligand after stage 6 of oogenesis [17, 48] and is subsequently cleaved by γ -secretase components Nicastrin [49, 50] and Presenilin [51], in order to bind the nuclear effector Suppressor of Hairless (Su(H)) and activate transcription of downstream targets. Notch remains localized in the apical domains of follicle cells during the early mitotic stages until activation, where antibody staining becomes diffuse in follicle cells [48]. The expression of three independent Notch reporter lines also reveals that Notch is active in the MbFCs during stages 7–10A; levels are highest at stage 8 [19, 39, 52, 53]. Removal of *Dl* function in the germ-line cells or *Notch* function in the MbFCs keeps follicle cells in the mitotic cycle and undifferentiated during midoogenesis, thereby connecting Notch activation with the induction of the M/E switch [17, 48]. Notch signaling regulates Stg and Dap negatively and Fizzy-related (Fzr), an adaptor for the APC/C E3 ligase, positively [15]. Downregulation of Stg and Dap allows CycE to oscillate and drive the endocycle. Activation of Fzr allows for the degradation of the mitotic cyclins, thereby ensuring complete transition into the endocycle [54]. Intriguingly, Stg and Dap are downregulated normally and Fzr is expressed at the end of stage 6 in follicle cells defective for JNK signaling [27], in which the unknown downstream targets of the JNK pathway are thought to be distinct from the known Notch-regulated targets.

Factors required for the endocycle switch

Notch-activated endocycle progression depends on expression of the Notch target *hindsight* (*hnt*). *hnt* encodes a zinc-finger transcriptional repressor that terminates mitosis and promotes the endocycle through downregulation of Cut [29]. *hnt* mutant cells show extended mitotic activity with continued expression of Cut, yet Notch activity remains unchanged [29]. Overexpression of Hnt before stage 6 results in larger nuclei and loss of mitotic cell-cycle markers, suggesting that its activation suffices to promote premature entry into the endo-cycle in follicle cells [29].

hnt also suppresses Hh signaling during the M/E transition, as shown through the *ptc*-LacZ reporter [29]. Elevated levels of *ci*-LacZ expression and Ci protein persist in *hnt* mutant follicle-cell clones during midoogenesis, demonstrating that Hnt regulates the Hh pathway through transcriptional repression of *ci* [29]. Hnt regulation of Hh signaling appears to be independent of PKA activity, because Pka regulates Ci protein degradation but not transcription [29]. *ptc* mutant cells overproliferate mildly; cells that do not receive Dl signaling from the germ line remain in mitosis and overproliferate [29]. Therefore, Hnt

appears to be a crucial factor governing the activation of the M/E switch and mediates Notch-dependent downregulation of Hh signaling in follicle cells.

Other genes required for the M/E switch include the transcription factor *tramtrack* (*ttk*), which expresses only one isoform (Ttk69) in follicle cells. Ttk69 is required for the M/E switch, and its expression is not affected by *Notch* or *hnt* LOF, suggesting that Ttk69 is not a Notch or Hnt target but works with Notch in a parallel pathway to promote the M/E switch [27, 29]. *ttk* mutant cells after stage 6 exhibit persistent *stg-lacZ* and *dap-5gm* reporter activity, with concurrent loss of Fzr-LacZ expression [27]. Like *Notch*, *ttk* LOF also upregulates *ci-LacZ* expression and Ci protein levels, demonstrating that Hh signaling is a common target of Notch and Ttk [29], but Ttk differs from Notch in regulating some cell differentiation markers: Fasciclin III (FasIII; early-oogenesis marker) expression is properly downregulated in *ttk* but not *Notch* midoogenic clones [27, 29]. *emc* is also needed for the M/E switch, as *emc* mutant cells remain in mitosis [44]. *Emc* expression is extremely reduced in follicle cells by stages 8–9, similar to that of *Eya* [44]. Although *emc* is a target of Notch signaling in follicle-cell precursors, whether *Emc* expression during the M/E switch is modulated by Notch signaling has not been directly tested. Overall, the evidence here supports the probability of additional Notch targets at the M/E switch, as well as parallel pathways that work in concert with Notch activation to promote cellular differentiation and the endocycle.

Follicle-cell growth and DNA rereplication during the endocycle

CycE, the major regulator of the endocycle in midoogenesis, also oscillates during early oogenesis; after the M/E switch, CycE protein levels are reduced but still oscillate [15]. Cells mutant for *archipelago* (*ago*), which marks phosphorylated CycE for degradation, show increased levels of CycE expression at almost all stages [15]. *ago* is dispensable for mitosis, but *ago* mutant follicle cells that survive past stage 6 continue to express mitotic cyclins and possess smaller nuclei, similar to follicle cells overexpressing CycE after stage 6 [15]. *Dap* expression during early oogenesis may account for the dispensability of *Ago*, in that, despite increased CycE levels, *Dap* is able to inhibit CycE activity and promote mitotic activity. What accounts for the reduction of CycE after the M/E switch is currently unknown; lower levels of CycE protein appear to be required for the transition because loss of *Ago* expression during mitosis impedes the M/E switch through accumulation of CycE and keeps the cell in a mitotic state [15].

Recent evidence also implicates the Insulin Receptor (InR)/Phosphatidyl 3-Kinase (PI3K) pathway in the promotion of the endocycle and follicle-cell growth. The InR/PI3K pathway regulates development through nutrient-sensing mechanisms that respond to environmental conditions. Activation of InR in current models promotes dAkt and Target of Rapamycin (Tor) kinase activity, in which dAkt promotes cellular growth through Tor [55]. Follicle-cell growth is particularly sensitive to kinase activity, as *dAkt* and *tor* LOF (and overexpression of the dAkt antagonist PTEN) result in smaller nuclei during midoogenesis, whereas overexpression of dAkt produces larger follicle-cell nuclei [56–58]. Although *dAkt* mutant cells past stage 6 terminate mitosis normally with reduced DNA content, *tor* follicle cells, for unknown reasons, cannot switch to the endocycle and remain mitotic [56]. In the case of cell growth, Foxo (an InR transcription factor) has been shown in *Drosophila* larval muscle to antagonize dMyc, a bHLH transcription factor involved in cell growth [59]. *dmyc* mutant flies have significantly smaller bodies than their wild-type siblings, and *dmyc* follicle cells can terminate mitosis but fail to undergo the endo-cycle efficiently [60]. dMyc regulation by the InR/Tor pathway may not be entirely canonical, as *foxo* mutations cannot rescue *InR* mutant phenotypes in the follicle cells, indicating that InR/Tor signaling operates without Foxo input in oogenesis [56]. Given that Tor is also regulated by amino acid and energy

sensing pathways [12], follicle-cell growth and endoreplication are probably regulated by the InR pathway, whereas the M/E switch itself may be regulated by insulin-independent Tor function. Although no direct evidence yet supports interaction between Notch and InR signaling at the M/E switch, insulin signaling has been shown to maintain the niche in the germarium by bridging Notch and E-cadherin to maintain cap-cell and germ-line stem-cell interactions [61], thus raising the possibility of further downstream interactions between InR and Notch.

Overall, the data on follicle-cell endocycle and growth show a trend in which Notch activation is needed, in conjunction with additional genes and pathways for phosphorylation and degradation of mitotic proteins, to prepare the cells to enter the endocycle. Regulation of CycE levels during the endocycle stages is crucial for follicle cell growth and disruption of these oscillations or pathways monitoring nutrient uptake/growth produces defective endocycling. In addition, clonal analysis in the JNK pathway shows that some of its targets required for the M/E switch might be independent of Notch activation [27]; further analysis is needed to determine their relationship with Notch signaling in follicle cells.

Transition to late oogenesis: The gene-amplification cycles

During late oogenesis, the egg chamber prepares the oocyte for eventual fertilization and deposition on a food source. At stage 10B, follicle cells over the oocyte switch to the third cell-cycle regimen, gene amplification (Fig. 5). Genome-wide DNA synthesis is inhibited except for genes encoding chorion proteins necessary for eggshell synthesis, as required to protect the embryo from environmental factors. Maximum production of these proteins is achieved through continuous rereplication of chorion genes without a resting gap phase, in contrast to the canonical endocycle, at a time when follicle-cell growth and migration over the oocyte is completed—the endocycle–gene amplification (E/A) switch.

Downregulation of Notch signaling at stage 10B is essential for the E/A switch; expression of NICD, the constitutively active form of Notch, after stage 10A causes follicle cells to undergo an extra round of the endocycle, with extended Hnt expression and loss of Cut reactivation [62, 63]. Down-regulation of Notch is required for upregulation of Ecdysone Receptor (EcR; hormone binding) activity at stage 10B in follicle cells [62, 64]. Expression of a dominant-negative form of the EcR-A isoform (EcRA-DN) results in loss of gene amplification and thin eggshells [64], persistent Hnt expression [62], and loss of Cut [62], demonstrating the importance of EcR signaling in the E/A switch.

In addition, levels of Ttk69 expression appear crucial for the E/A switch. As mentioned previously, Ttk69 is present throughout the majority of oogenesis at low levels. As Notch downregulation begins, Ttk69 levels are dramatically upregulated, a process that also requires EcR activity. Ttk mutant clones cause cells to fail to undergo gene amplification, whereas Ttk overexpression results in premature exit from the endocycle without entry into gene amplification [62]. RNAi studies show that Ttk69's role in the E/A switch is independent of its earlier involvement in the M/E switch [62]. Overexpression of Ttk69 can alleviate the endocycle-exit defects caused by extended Notch activity or removal of EcR function [62], suggesting a regulatory relationship between these two pathways and *ttk* during the E/A switch.

After the E/A switch, CycE expression becomes uniform, and BrdU incorporation is restricted to chorion-gene loci [19, 62]. Although BrdU incorporation patterns and uniform CycE expression imply that the cell cycle is synchronous during gene amplification, whether every follicle cell amplifies at the same rate is not known. The formation of eggshell structures may require more chorion protein in distinct areas for proper development. As

discussed below, a possible mechanism for influencing chorion protein synthesis rates may involve chromatin remodeling through histone acetylation of chorion origins.

Histones H3 and H4 acetylation levels rapidly increase in concurrence with binding of the amplification complex to promote replication of DNA at chorion-gene loci [65, 66]. Transcription factors E2F1/DP and Myb/MuvB both participate in modulating chromatin structure to allow or repress DNA replication at chorion-gene loci. Under conditions allowing origin firing, the E2F1/DP binding partner Rbfl is phosphorylated by CycE, physically associates with Myb/MuvB complexes bound on chromatin, and promotes histone acetyltransferase activity. In contrast, unphosphorylated Rbfl dissociates from Myb/MuvB and interacts with E2F1/DP, forming a repressor complex that recruits the histone deacetylase Rpd3 to remove histone acetylation marks and prevent DNA synthesis [65]. Intriguingly, small stage-10B Rpd3 mutant clones induce genome-wide hyperacetylation and redistribution of the Origin Recognition Complex 2 (ORC2) replication protein, suggesting that inappropriate DNA replication is occurring [67]. Further work involving a special in-vivo tethering assay modulates chorion protein synthesis by directing Rpd3 and Polycomb proteins to suppress histone acetylation at chorion-gene loci and Chameau (Chm) histone acetyltransferase to increase it [67]. What allows phosphorylation of Rbfl by CycE only at chorion origin sites is unknown, but nevertheless chromatin remodeling appears to function prominently in gene amplification.

Before the end of oogenesis, a final round of cellular differentiation occurs simultaneously when gene amplification is underway. The Notch pathway is required along with input from the JNK and epidermal growth factor receptor (EGFR) pathways for extensive remodeling of follicle cells into three specialized eggshell structures: the dorsal appendages, the micropyle, and the operculum. In particular, Puc is required for the expression of AP-1 transcription factors dFos and dJun, whereas Bun expression maintains boundaries of Notch signaling to aid in correct morphogenesis of these structures [68, 69]. The extent to which gene amplification and signaling pathways influence each other is not well understood. An intriguing study reports interactions between Chm and JNK signaling in regulation of epithelial morphogenesis during *Drosophila* thorax closure by phosphorylation of dFos to allow transcription of Chm-bound downstream targets, whereas conditions that promote dephosphorylation of dFos recruit Rpd3 to repress transcription [70]. Whether this pathway is also active during late *Drosophila* oogenesis is unknown, but given that dFos and dJun are highly elevated in anterior follicle cells [71], interactions between JNK signaling and chromatin remodeling may have significant roles in epithelial morphogenesis.

Cell-cycle regulation in specialized follicle cells

Specialized PCs and stalk cells contribute to proper egg-chamber development and polarity. At regions 2a/b in the germarium, some FSC daughter cells are converted into cross-migrating cells (cmcs) and posterior-migrating cells (pmcs), which migrate to the anterior and posterior ends of the germ-line cells (Fig. 2B) [21, 72]. They are initially specified as PCs, and additional PC specification occurs during R3 [21]. After the divisions, the PCs all undergo cell-cycle arrest at G2 phase through downregulation of Stg [73]. As the egg chamber leaves the germarium, JAK/STAT signaling interfaces with Notch/Dl signaling to specify other precursor cells as stalk cells with the help of *fringe* (*fng*), a glycosyltransferase that increases Notch signaling and also stops mitotic activity [39]. By stages 4–5 of oogenesis, the initial four or five PCs at each end of the egg chamber are reduced to two pairs at the termini by apoptosis [74].

Overexpression studies have also yielded insight into the nature of PC specification and cell-cycle regulation. Although PCs undergo cell-cycle arrest, they can be induced to undergo

mitosis or the endocycle, as evidenced by misexpression of *Stg* and *Fzr*, respectively, with the PC-specific *upd*-GAL4 driver. In contrast, misexpression of NICD with the same *upd*-GAL4 driver does not produce any distinct phenotype. Conversely, misexpression of NICD with the *c306*-GAL4 driver, which is expressed at the termini of the egg chamber, results in ectopic PC formation. Because Notch activity is normally present only in PCs, stalk cells, and their precursors before stage 7 [39], activation of Notch in the terminal follicle cells surrounding the endogenous PCs somehow recruits these to a PC fate [73]; the ectopic PCs are not due to defects in apoptosis-mediated cell death, as a subset of these cells express activated Caspase-3, which marks cell death [73].

The mechanism of PC specification is still poorly understood, because gene expression in PCs, including *Cut*, is largely observed after *cmcs*/*pmcs* have migrated. Given that Notch appears to be active before *Cut* in PCs, *Cut* could be a Notch target similar to unidirectional Notch signaling in the dorsoventral boundary of the wing imaginal disc, in which cells undergo G1/G2 arrest [75–78]. Along with *Notch* [79, 80], PCs express a subset of genes such as *neuralized* [45, 81], and *upd* [82, 83], that are also expressed in the nervous system and sensory organs, tissues that require cell cycle arrest for correct cell specification [84]. Thus, PC specification appears to be contingent on Notch activation and transcription of pro-neural genes (a subset of which is not expressed in any other cells during oogenesis [45, 82]), which may be the deciding factor as to whether the cell-cycle is promoted or arrested.

Proliferation defects in tumor suppressor pathways

Recent research has focused on elucidating the functions of tumor-suppressor genes in the control of cellular proliferation in mammalian and *Drosophila* development. Multiple pathways and genes are involved in tumor suppression, including *retinoblastoma factor 1* (*rbf1*), *p53*, the hyperplastic Hippo pathway, and the neoplastic nTSG genes, which encode endocytic components and scaffold/cell-cell junction proteins. Cells with mutations in the Hippo pathway components proliferate during midoogenesis through aberrant regulation of the transcription factor Yorkie [85–88], which affects cell-cycle regulation without defects in tissue architecture. nTSG genes that encode proteins involved in cell-cell junctions and scaffolding affect epithelial integrity through loss of apical-basal follicle-cell polarity [89, 90]. Mutations in Hippo components and nTSG genes both confer loss of PFC cell-fate determination (as seen through EGFR reporters at stages 6–7 of oogenesis [86, 90]) and failure to enter the endocycle and cause cells to remain in a proliferative state. JAK/STAT signaling remains largely intact in both Hippo pathway [87] and *discs large* (*dlg*)/*scribbled* (*scrib*) mutations [90], suggesting defects in signaling pathways occur independently of JAK/STAT activity. Further analysis with endocytosis markers reveals defects in general endocytosis in Hippo pathway mutants, which result in accumulation of Notch at the apical surface of the follicle cells and loss of Notch signaling [86]. In contrast, *dlg*/*scrib* mutant clones that are adjacent to the germ line are able to activate Notch; the defects in apical-basal polarity in these clones appear to impede coordination of JAK/STAT, EGFR, and Notch signaling, resulting in tumorous growth [90].

The other distinct group of nTSG genes that encode components involved in endocytosis also affects Notch signal processing [91, 92]. After cleavage, NICD progresses through early and late endocytic compartments and multivesicular bodies on its way to the nucleus, which subjects it to a variety of processing mechanisms [93]. Impairment of proteins that form early endocytic components blocks Notch entry into early endosomes and leads to loss of Notch activation [94, 95]. Conversely, mutations in late endocytic components maintain Notch localization in the endosomes, which result in accumulation and increased Notch signaling [96]. More studies are also needed to determine whether the phenotypic defects of mutations in nTSG cell-architecture components are independent of endocytic mechanisms.

In addition, defects in endocytosis probably affect processing of multiple ligands, and more research should be conducted to determine whether other signals work in concert with Notch to regulate cell differentiation and proliferation in follicle cells. On the whole, the evidence presented here points to multiple levels of tissue regulation that can affect the cell cycle, cell polarity, and signal transduction in follicle cells and promote conditions favorable for tumorigenesis.

Conclusions and perspectives

Examination of the research conducted in *Drosophila* oogenesis reveals several themes with regard to cell cycle and cellular proliferation. In the vicinity of Notch activation, the mitotic follicle cells expend many resources in preparation for the switch to the endocycle and proper downregulation of mitotically active genes. Given the different types of genes needed for endocycling and follicle-cell growth, other Notch targets and parallel pathways working in concert to regulate these processes remain to be discovered. Furthermore, an increasing amount of evidence shows that endocytic processing of Notch (and possibly other ligands) is just as important for promotion of timely cell-cycle changes and cellular differentiation by signal transduction pathways.

Defects in the M/E and E/A switches demonstrate that tumorigenesis is a result of dysregulation of multiple signaling pathways, including Notch and JAK/STAT, which ultimately result in uncontrolled growth. Despite the simplicity of the *Drosophila* oogenesis system, the mechanisms promoting the minimal conditions for tumorigenesis still remain unclear, but the studies of tumor-suppressor mutations do imply that impedance of signaling pathways (rather than complete obliteration) lead to a lack of coordination in signaling pathways and a subsequent potential for tumorigenesis to occur.

In addition, comparison of PCs and MbFCs also reveals that spatiotemporal differences in expression of genes common to the two cell types can induce dramatic differences in their cell-cycle programs and cellular identity, which may prove useful in finding future therapeutic targets to stall and/or eliminate cancerous cells. Although research to date has revealed a great amount of detail about cell-cycle changes and cellular differentiation in *Drosophila* oogenesis, much work remains to be done in elucidating how these pathways interact with each other. Such work will undoubtedly yield better understanding and potential strategies for deterring tumorigenesis in mammalian cancers.

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Abbreviations

APC/C	anaphase promoting complex/cyclosome
AFC	anterior follicle cell
bHLH	basic helix-loop-helix
BrdU	bromodeoxyuridine
cmc	cross-migrating cell
DAFC	<i>Drosophila</i> follicle cell amplicons
E/A	endocycle-gene amplification

EGFR	epidermal growth factor receptor
FLP	flipase
FRT	flipase recognition target
FSC	follicle stem cell
GOF	gain of function
H3	histone 3
H4	histone 4
JAK	janus kinase
JNK	c-Jun N-terminal kinase
LOF	loss of function
MbFC	main-body follicle cells
M/E	mitotic cycle-endocycle
nTSG	neoplastic tumor suppressor gene
PH3	phosphohistone 3
PFC	posterior follicle cell
pmc	posterior-migrating cell
R1	region 1
R2a/R2b	region 2a/b
R3	region 3
UAS	upstream activation sequence

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Figure 1. Schematic of *Drosophila* oogenesis. In the *Drosophila* ovariole, the egg chamber progresses through 14 distinct stages before fertilization and deposition on a food source. Legend: s, stage; A, anterior; P, posterior; nc, nurse cell; oo, oocyte; da, dorsal appendage; op, operculum.

Figure 2.

Cross-migrating cells (cmcs) and posterior-migrating cells (pmcs): migration and initial specification of the polar cells. **A:** The niche induces germ-line stem cells to divide and create the cystoblast, which undergoes multiple divisions with incomplete cyto-kinesis to create a 16-cell cystocyte, in which one nucleus becomes the oocyte and the other 15 become nurse cells. Somatic stem cells proliferate to create follicle cells that will encapsulate the egg chamber; some of these follicle cells differentiate further to become polar cells and interfollicular stalk cells that connect the older egg chamber to its younger antecedent. **B:** During R2, Delta signaling from the germ line transiently induces Notch activation in a cmc from the somatic stem cell, which leads to migration of the cmc to the anterior of the cystocyte. An unknown signaling event induces the pmc from the alternate somatic stem cell to migrate to the posterior of the cystocyte. By R3, multiple polar cells are specified from the cmcs and pmcs. TF, terminal filament; CC, cap cells; ESC, escort stem cells; GSC, germ-line stem cells; IGS, intergonial sheath; CB, cystoblast; CS, cystocytes; SSC, somatic stem cell; FC, follicle cell; Oo, oocyte; SC, stalk cells.

Figure 3.

Schematic drawing of different follicle-cell fates upon stepwise activation of multiple signaling pathways. **A:** In this stage-5 egg chamber, all follicle cells except the polar cells undergo the mitotic cell cycle before the M/E switch. Cut is expressed in both main-body follicle cells (MbFCs) and the polar cells, whereas Notch activation is expressed everywhere. In addition, Upd expression in the polar cells activates the JAK/STAT pathway and maintains the anteroposterior axis polarity of the egg chamber. **B:** In this stage-8 egg chamber, Notch is expressed in all follicle cells; addition of the EGFR pathway along with JAK/STAT facilitates induction of posterior follicle-cell (PFC) fate at the posterior of the egg chamber, and anterior follicle cell (AFC) fate is designated by both Notch and JAK/STAT. **C:** In this stage-10B egg chamber, upregulation of EcR and Ttk69 allows gene amplification to occur in the chorion gene loci. At this stage, H3 and H4 acetylation is

upregulated at the chorion gene loci, as is assembly of the amplification complex to facilitate transcription of eggshell genes.

Figure 4.

A model of multiple pathways inducing the switch from mitosis to the endo-cycle. This transition is initiated by binding of Delta to Notch, which promotes intracellular cleavage of Notch and binding to Su(H) for transcription of downstream targets. Although expression of JNK components ceases after the switch to the endocycle, the persistent expression of Ttk69 implies that the presence of Ttk69 is required to facilitate the switch, whereas JNK signaling actively promotes mitosis through regulation of the cell cycle. Notch activation upregulates Hindsight expression, which then downregulates Cut expression in the presence of Polycomb group members Psc and Su(z)2, as well as Stg (whether Hnt downregulates Dap after Notch activation is unknown). Loss of Cut expression relieves repression of Fzr, which degrades mitotic cyclins and promotes the endocycle in the midst of String and Dacapo expression by Notch. Ago degrades Cyclin E in the mitotic and endocycle phases but is dispensable during mitosis; after downregulation of Dacapo by Notch, Ago is required to maintain CycE levels during the endocycle oscillations. In addition, an unknown sensing

pathway (X) activates Tor expression to modulate the switch to the endocycle; activation of dAkt by the Insulin Receptor pathway also upregulates Tor to promote endoreplication and follicle-cell growth. Connections implied by research data but lacking direct evidence are denoted by dotted lines.

Figure 5.

A model of pathways involved in gene amplification downregulation of Notch to residual levels at stage 10B is coincident with a high rate of EcR activity, which in turn upregulates Ttk69 levels and promotes the switch from the endocycle to gene amplification. CycE expression changes from an oscillating pattern to high, sustained levels, disassociating Rbf from the repressive E2F/DP complex via phosphorylation and allows histone acetylation by a histone acetyltransferase (HAT) and assembly of the amplification complex (AC) and the origin recognition complex (ORC) to initiate origin firing.

Table 1 Genes involved in cell cycle/cell proliferation in *Drosophila* oogenesis

	Expression/localization	LOF defect – follicle cell clones	Refs.
<i>hh</i> (Hh)	TF, CC	Loss of FSC maintenance and division	[23, 28, 31–34]
<i>ptc</i> (Hh)	MbFC S1-6	Mitotic proliferation, high Ci expression	
<i>pka</i> (Hh)	MbFC S1-6	Higher Ci expression S1-6	
<i>ci</i> (Hh)	MbFC S1-6	No endocycling	
<i>wg</i> (Wg)	TF, CC	Loss of FSC maintenance	[25–27]
<i>sgg</i> (Wg)	MbFC S1-6	No endocycling, Notch high at st10A	
<i>axin</i> (Wg)	MbFC S1-6	No endocycling	
<i>dsh</i> (Wg)	MbFC S1-6	VA135 – normal, 477 – divides slowly	
<i>upd</i> (JAK/STAT)	PC	Defective AP axis	[37–40]
<i>hop</i> (JAK/STAT)	gradient at termini	Defective AP axis	
<i>ralA</i> (JAK/STAT)	PC	Loss of PC and defective AP axis	
<i>notch</i> (Notch)	PC, MbFC S7-10A	No endocycling	[27, 29, 39, 42, 62]
<i>cut</i> (Notch)	PC, MbFC S1-6, DAFC	Early M/E switch	
<i>hnt</i> (Notch)	MbFC S7-10A	No endocycling	
<i>Dl</i> (Notch)	GE, MbFC	No endocycling (GE), no anterior PC (FC)	
<i>fng</i> (Notch)	PC, SC	No SCs or PCs	
<i>bsk</i> (JNK)	MbFC S1-6	Early M/E switch	[27, 71]
<i>hep</i> (JNK)	MbFC S1-6	Defective mitosis	
<i>puc</i> (JNK)	MbFC S1-6	No endocycling	
<i>tor</i> (InR)	MbFC S1-10A	No endocycling	[56–58]
<i>dAkt1</i> (InR)	MbFC S1-10A	Defective endocyclic follicle-cell growth	
<i>EcR</i> (EcR)	BC, DAFC	Defective E/A switch	[27, 62]
<i>ttk69</i> (EcR)	S1-10A low, DAFC high	No endocycling (M/E, E/A)	
<i>bun</i>	DAFC subset	Defective DA and operculum formation	[68, 69, 71]
<i>dJun</i>	DAFC subset	Defective DA and operculum formation	
<i>dFos</i>	DAFC subset	Defective DA and operculum formation	
<i>E2F1/DP</i>	S1-10A oscillating, DAFC uniform	Loss of gene amplification	[65–67]
<i>rbf1</i>	DAFC	Loss of gene amplification	
<i>myb/muvB</i>	DAFC	Loss of gene amplification	
<i>rpd3</i>	S1-14	Genome-wide hyperacetylation in DAFC	
<i>eya</i>	MbFC S1-7, no PC	MBFCs become PCs	[44, 45]
<i>emc</i>	MbFC S1-7, PC st4-14	MBFCs become PCs	
<i>hippo</i> (Hippo)	MbFC S1-6	Proliferation at posterior, Notch LOF	[85–88]
<i>sav</i> (Hippo)	MbFC S1-6	Proliferation at posterior, Notch LOF	
<i>wt5</i> (Hippo)	MbFC S1-6	Proliferation at posterior, Notch LOF	
<i>kibra</i> (Hippo)	MbFC S1-6	Proliferation at posterior, Notch LOF	
<i>dlg</i>	FC – apico-basal	Proliferation at termini	[89, 90]

	Expression/localization	LOF defect – follicle cell clones	Refs.
<i>scrib</i>	FC – apico-basal	Proliferation at termini	
<i>l(2)gl</i>	FC – apico-basal	Proliferation at termini	
<i>avl</i>	FC – early endosome	Notch LOF	[91–96]
<i>rbsn</i>	FC – early endosome	Notch LOF	
<i>shiDN</i>	FC – early endosome	Notch LOF	
<i>rab5</i>	FC – early endosome	Notch LOF	
<i>tsg-101</i>	FC – late endosome	Notch accumulation (GOF)	
<i>vps-25</i>	FC – late endosome	Notch accumulation (GOF)	
<i>vps-20</i>	FC – late endosome	Notch accumulation (GOF)	

CC, cap cells; DAFC, *Drosophila* follicle-cell amplicons; FC, follicle cells; GOF, gain of function; GE, germ-line cells; LOF, loss of function; MbFC, main-body follicle cells; PC, polar cells; SC, stalk cells; TF, terminal filament.