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Polyploidy in development and tumor models in *Drosophila*

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Abstract

Polyploidy, a cell status defined as more than two sets of genomic DNA, is a conserved strategy across species that can increase cell size and biosynthetic production, but the functional aspects of polyploidy are nuanced and vary across cell types. Throughout *Drosophila* developmental stages (embryo, larva, pupa and adult), polyploid cells are present in numerous organs and help orchestrate development while contributing to normal growth, well-being and homeostasis of the organism. Conversely, increasing evidence has shown that polyploid cells are prevalent in *Drosophila* tumors and play important roles in tumor growth and invasiveness. Here, we summarize the genes and pathways involved in polyploidy during normal and tumorigenic development, the mechanisms underlying polyploidization, and the functional aspects of polyploidy in development, homeostasis and tumorigenesis in the *Drosophila* model.

Keywords

polyploidy; cancer; development; drosophila; endoreplication; endocycle

Introduction

Polyploidy is relevant to specific tissues and their development and is a strategy used by cells to increase cell size and transcriptional output in eukaryotes [1–4]. Cells increase their ploidy level by a variety of processes, including acytokinetic mitosis, cell fusion, endomitosis and endocycle (Fig. 1). In cell fusion, cells merge to form a single multinucleated cell; for example, myoblasts fuse and form polynucleated muscle fibers [2, 5–7]. Acytokinetic mitosis is associated with failure of cytokinesis: cells undergo all cell cycle stages (G1-S-G2-M) but cytokinesis fails to occur, which results in a multinucleated

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Conflict of interest

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cell [2, 5, 7]. Endomitosis involves a partial mitosis in the absence of both karyokinesis and cytokinesis, as seen in megakaryocytes [2, 5]. A fourth mechanism for increasing ploidy is called the endocycle, where mitosis is entirely evaded and genomic DNA is doubled or nearly doubled between an intervening gap (G) phase [2, 5–7]. Endoreplication refers to replication of the genome via actyokinetic mitosis, endomitosis or the endocycle. In *Drosophila*, the endocycle can produce polytene chromosomes, which are a fusion of sister chromatids that have not undergone segregation. These chromosomes have densely packed heterochromatin interspaced with loosely packed euchromatin, forming distinct banding patterns visible with electron microscopy [8, 9]. Because the endocycle can increase DNA content without increasing the chromosome set number (N), the ploidy of polytene cells is measured in multiples of the total DNA content in a gamete (C). In this review, we will discuss studies that have used *Drosophila melanogaster* as a model system to uncover the intricacies that promote polyploidization, primarily focusing on the endocycle and its role in normal and tumorigenic tissues.

Polyploidy affects development by impacting organ size and function. In mammals, cytokinetic failure frequently occurs soon after fertilization. The resulting polyploid cells exist as multinucleated blastomeres in the developing embryo [10–13]. Polyploidization is also inherent to the developing placenta, as villous trophoblasts fuse to form multinucleated syncytiotrophoblasts and as trophoblast stem cells differentiate by endoreplication into trophoblast giant cells, which are vital for placenta growth, implantation of the conceptus into the uterus, lactogenesis, regulation of maternal blood flow, and synthesis of cytokines and hormones like progesterone [14–16]. Polyploidization is also seen in megakaryopoiesis, where megakaryoblasts perform endomitosis to mature into megakaryocytes. Polyploidy in megakaryocytes is supportive of large cell size and high transcriptional output appropriate for platelet biogenesis [17–19]. In liver parenchyma, stochastic cytokinetic failure of cells occurs during postnatal growth, patterning hepatic tissue with mosaic ploidy levels [20, 21]. In various human solid tumors, histopathologists have recognized Polyploid Giant Cancer Cells (PGCCs), which can be identified by a comparatively large nucleus size and cell size three times or greater than regular cancer cells [22, 23]. Additionally, the high prevalence of polyploidy in tumors has been inferred from clinical sequencing data of several thousands of cancer patients [24]. The occurrence of genomic doubling is closely associated with worse prognosis, making the presence of polyploidy a predictor for patient survival and tumor evolution [25]. Genomic doublings and PGCCs appear early in tumorigenesis, implying a role for tumor initiation. Polyploidy in human tumors have also been associated with drug resistance and metastasis [22, 26–28].

Drosophila is a well-established model to study polyploidy as polyploid cells exist across distinct organs such as the ovary, gut, brain, and salivary glands [29–33]. Besides this advantage, the *Drosophila* model also includes more than a century of research, and a great number of genetic tools and techniques are therein described [34]. These advantages set *Drosophila* apart as a leading model to explore genes and mechanisms involved in the regulation of polyploidization particularly via endoreplication. In line with this argument, this review focuses on polyploidization primarily in reference to endocycling *Drosophila* cells. The first section starts off by describing the various instances of polyploidy during *Drosophila* development. The middle sections discuss distinct genes and pathways that

influence the endocycle in different tissues. The review concludes with a discussion of polyploidy on wound repair and tumorigenesis.

1. Polyploidy in *Drosophila* development

Polyploid cells are present across a wide variety of organs and tissues in *Drosophila* and are found throughout all four stages of the insect's holometabolous life cycle, comprising embryo, larva, pupa, and adult. These polyploid cells have assorted features and functions.

In the embryo, DNA is endoreplicated through endocycles between 8 and 15 hours after fertilization at specific times and domains of the salivary gland, Malphigian tubules, and gut [29, 35]. Polyteny is a property of these cells and refers to chromosomes formed as a fusion of undivided chromatids [36]. In the salivary gland, the polytene cells include the secretory cells, which undergo asynchronous endoreplications at embryogenesis to become giant cells [33].

In *Drosophila* larvae, large size and ample DNA content of the salivary gland cells is appropriate for the synthesis and secretion of digestive enzymes and adhesive substance for attachment to the substrate. Polytene cells of the Malpighian tubules remove toxic chemicals and nitrogenous waste from the hemolymph [37], making these structures analogous in function to the human kidney [38, 39]. *Drosophila* larvae also contain polytene cells in regions of the fat bodies, epidermis, trachea, brain, and prothoracic glands [29, 40–43]. The larval prothoracic glands regulate growth and maturation by secreting the steroid molting hormone ecdysone [44]. Ecdysone synthesis is endocycle-dependent and essential for larval development; inhibition of the endocycle in prothoracic gland cells reduces ecdysone synthesis and results in larval developmental arrest [45].

Many larval cells and tissues that are no longer useful are removed by programmed cell death at metamorphosis [46]. The hormone ecdysone contributes to this process by inducing histolysis of cells in the ileum, midgut, ventral nerve cord, anterior and abdominal muscle, optic lobe, and salivary gland [47–54]. During pupation, other modifications that concern or give rise to polyploid cells take place as well. For example, though senescence usually follows endoreplication, rectal papillar cells re-enter mitosis after having undergone endoreplication during the larval stage, making these cells susceptible to chromosome abnormality [48]. Additionally, cells of the sensory organ lineage such as the socket and shaft cells that form the hair bristles will undergo endocycles, with the size of the hair shaft dependent on the number of endoreplications [55].

In the adult, polyploid cells are included in the ovary, spermatheca, Malpighian tubules, gut and brain. The cell cycle program of the ovaries and the timing of endoreplication are well characterized. The somatic follicle cells surrounding germline cells form egg chambers that show distinct cell cycle programs during oogenesis, including the mitotic cycle (stages 1–6), the endocycle (stages 7–10a), and chorion gene amplification (stages 10b-13). During the endocycle phase, main body follicle cells undergo three rounds of endoreplication, an essential step that promotes polyploidy and cell growth [56–60]. Nurse cells also undergo multiple rounds of endoreplication. At stage 6, the nurse cell polytene chromosomes disassemble via a mitotic-like process into 32 pairs of chromatids that disperse throughout

the nucleus. Oogenesis proceeds with subsequent endoreplications, increasing nuclear size and leading to a final cell ploidy of around 1500C [61]. Nurse cells support oocyte growth by providing mRNA, proteins, and organelles through specialized ring channels [62-64]. Included within the female reproductive tract is the spermatheca, which is lined with polyploid secretory cells. These secretory cells contribute to large-scale bioproduction of secretory discharge, which helps with attraction of sperm to the sperm receptacle and regulation of ovulation [65–67].

During adult gut development, intestinal stem cells and enteroblasts require Notch signaling to decrease cell proliferation and to initiate differentiation into mature polyploid enterocytes [68, 69]. In the brain, neurons and glia are diploid at eclosion, except for the subperineurial glia, which undergo endocycles and endomitosis in the larval and pupal stages to form the blood-brain barrier. As the adult ages, several of the diploid cells acquire polyploidy as well, which serves as a protective effect against DNA damage-induced cell death [32].

2. Genes and pathways involved in polyploidy regulation

2.1 The cell cycle machinery

The cell cycle alternates among four sequential phases (G_1-S-G_2-M). Phase progression and transitions are correlated with the concentration of ancillary cyclins, which are regulatory proteins that form active complexes with cyclin-dependent kinases (CDKs). Cyclin concentrations oscillate while CDK concentrations remain relatively constant, providing temporal occurrence of cyclin-CDK complexes. These complexes activate or inhibit substrates by phosphorylation, catalyzing the cascade of events that lead to phase progression or transitions [70–72]. Cyclins are promoted for degradation by enzymes such as Fizzy-related (Fzr), which is auxiliary to the E3 ubiquitin ligase Anaphase Promoting Complex/Cyclosome (APC/C) [73, 74]. Activity of Cyclin-CDK complexes can further be regulated by cyclin-dependent kinase inhibitors like Dacapo (Dap). With specific expression of cell cycle regulators, the cell can bypass the M phase and enter the endocycle (M/E switch). Differential expression of regulatory factors allows for unique variation on endocycle progression.

CycE—CycE is the primary cyclin driving the endocycle. In contrast, mitotic cyclins are expressed at near undetectable levels throughout the endocycle, and while cyclin D is expressed during endocycles, it is primarily involved in cell growth, thus only affecting the endocycle indirectly [75]. Therefore, the endocycle implements CycE fluctuations in a relatively static backdrop of Cdk2 without much convolution by additional cyclins or CDKs [75].

Low levels of CycE-Cdk2 complex are correlated with DNA replication licensing during G phase because of a repressive role of CycE-Cdk2 on the pre-replication complex (pre-RC). Specifically, high levels of CycE-Cdk2 suppress pre-RC formation in part through Fzr inhibition, leading to Geminin (Gem) accumulation, as demonstrated in the salivary gland [74, 76–78]. Accordingly, constitutive expression of *cycE* affects endocycles by inhibiting DNA licensing and causing a reduction of DNA content of salivary gland cell nuclei. The role of *cycE* in mitotic cycles differs, as mitotic cycles were largely unaffected by

constitutive *cycE* expression [76, 77, 79]. CycE-Cdk2 is also required for DNA origin firing and progression through S phase [76, 80–82]. Ectopic expression can induce pre-mature S phase initiation. Furthermore, CycE regulates S phase duration, as its diminished expression heralds the completion of DNA replication. This role has been demonstrated in germline Nurse cells carrying a hypomorphic allele of *cycE* that shows persistent expression at late S phase. These mutant nurse cells have a lengthened S phase and ectopic replication of satellite DNA, these regions being normally under-represented in the wild-type [82].

Given that periodic activity of CycE-Cdk2 alternates the endocycle through DNA replicative and dormant phases, inputs and processes are maintained to regulate the activation and inactivation of this complex. CycE is embedded in an autoregulatory loop, with high *cycE* expression being a threshold for initiating its degradation [82]. Apontic and E2f1 have been shown to initiate G phase transcription of *cycE*, while CycE destruction is mediated by the Skp/Cullin/F-box (SCF) ubiquitin ligase complexed with Archipelago, the F-box specificity component [79, 83–85]. In the ovary and salivary gland, in addition to CycE oscillation, CycE-Cdk2 dynamics are reinforced by oscillatory expression of Dap, the CycE-Cdk2 inhibitor [86, 87].

CycA and CycB—CycA and CycB promote mitosis and progress the canonical cell cycle and are expressed in proliferating cells throughout development [88]. CycA forms complexes with several CDKs, and the CycA-Cdk1 complex likely drives mitosis through phosphorylation of the Myb-MuvB complex, which subsequently activates the transcription of M phase genes [89]. In the Schneider D2 (S2) cell line, knockdown of CycA, but not CycB, was sufficient to induce endoreplication [89]. Additionally, CycA knockdown in follicle cells induced M phase evasion and precocious entry into the endocycle [90].

Despite its role in promoting mitosis, CycA has been detected at low levels of oscillation in endocycling cells. This low level of expression is not entirely insignificant, as loss of CycA localized to cells of the bristle lineage and salivary gland decreases final ploidy of the fully differentiated cells. As a possible explanation, subnuclear re-localization of Orc2, a member of the Pre-RC complex, is dependent upon the minimal accumulation of CycA at mid S phase. The perturbance on timing of early and late S phase, where euchromatin and heterochromatin are replicated, respectively, may lead to a reduction of final cell ploidy [55].

Fzr—Fzr, the equivalent of the Cdh1 in mammals, is a positive regulator of APC/C, which targets and ubiquitinates M-phase components and cyclins such as CycA and CycB for degradation [91]. In the adult pylorus, Fzr mediates endocycle entry of cells neighboring empty spaces left by cell loss after injury, and endocycle entry leads to protection against tissue architectural and permeability disruptions [92]. In follicle cells, Notch signaling induces Fzr, which promotes the degradation of CycA and CycB, promoting cell transition from the mitotic to endoreplication cycle [59]. Similarly, in the embryonic salivary glands and midgut, Fzr is required to promote the degradation of CycA and CycB, ending the proliferative phase and beginning the endoreplication phase [73].

Dap—Dap protein, a cyclin kinase inhibitor with a similar binding-domain and function to p21 and p27 (mammalian homologs), specifically suppresses the CycE-Cdk2 complex [93].

In the embryonic epidermis, eye disc, and nervous system, Dap serves an essential role as an S phase inhibitor, and transient high expression of Dap coincides with permanent cell cycle arrest at G0 [93, 94].

Oscillatory Dap expression is required for proper endocycle progression in the ovary [86], and its oscillation is also needed for normal progression of the endocycle in the salivary gland [87]. Dap facilitates replication licensing by inhibiting CycE-Cdk2 activity at G phase [86]. Dap can also suppress CycE-Cdk2 activity during late S phase, which contributes to early S-phase truncation, meaning DNA replication is often incomplete during a normal endocycle, particularly in heterochromatic regions [86, 87]. In both the ovary and salivary gland, oscillatory Dap expression is due to S phase-dependent ubiquitylation of Dap by the E3 ubiquitin ligase $CRL4^{Cdt2}$. Dap has a PIP degron region that binds to DNA-bound PCNA, the sliding clamp that is active during DNA replication, and preferential Dap ubiquitination occurs when Dap is bound to DNA-bound PCNA [87, 95].

Cdc6—Cdc6 serves as a primary DNA replication licensing factor. Cdc6 together with Cdt1/Double-parked (Dup) initiates DNA replication via recruitment of the replicative helicase MCM2–7 and other initiation factors to the origin recognition complex (ORC), forming the pre-RC [96]. In this context, polyploid cells, which require multiple rounds of DNA replication, are reliant upon Cdc6 for maturation. Indeed, loss of function studies confirmed that Cdc6 is pertinent to the onset of DNA replication in both diploid and polyploid cells; quantitative analysis by flow cytometry on fat body and follicle cells with loss-of-*Cdc6* shows significantly decreased levels of ploidy [97]. Additionally, in the adult central nervous system, where glia and mature neurons exhibit high levels of ploidy as a cushion for accumulative DNA stress, knockdown of Cdc6 inhibits or reduces polyploidy [32].

For proper DNA replication during either the mitotic cycle or endocycle, licensing is only initiated once. While evidence using yeast cells suggests that Cdk complexes phosphorylate Cdc6 and inhibit re-licensing [98], in *Drosophila*, a more significant factor to re-licensing inhibition is negative regulation by Geminin, which can bind and sequester Dup [99, 100].

2.2 Transcription factors

Transcription factors (TF) are essential regulators of gene expression, making them essential to cancer and organ development. Some TFs display oncogenic activity and are targets of drugs in cancer therapies [101, 102]. Many functional aspects of *Drosophila* TFs are conserved in humans. For example, overexpression of Myc is able to enhance endoreplication in p53 mutated melanoma cells that were M-phase arrested by the damaging agent paclitaxel [103]. And in *Drosophila* follicle cells, overexpression of *Myc* promotes extra rounds of endoreplication and increases nuclear sizes [85, 104, 105]. This section reviews relevant studies that involve *Drosophila* TFs involved in polyploidization of cells.

E2f1—E2f1, an evolutionarily conserved TF from the E2f family, is required for S phase entry in both mitotic and polytene tissues [106]. Specifically, E2f1 forms a transcriptional heterodimer with Dp to regulate genes involved in the G1 to S phase transition such as *CycE*, *PCNA* and *RnrS* [82, 107–109]. E2f1 protein oscillates during endocycling salivary

gland cells, which contrasts with the ubiquitous expression of *e2f1* mRNA. Stabilized E2f1 blocks DNA replication by deregulating genes such as *CycE*, *Cdk1* and mitotic cyclins [79]. E2f1 levels are high at G phase, causing CycE expression and entry into S phase. In the salivary gland, S phase entry is delayed by the second isoform of E2f1, E2f1b, which upregulates Dap [110]. Upon entry into S phase, $CRL4^{Cdt2}$ ubiquitin ligase mediates E2f1 degredation, which is coupled to DNA synthesis by interaction of E2f1 PIP box with PCNA [111]. PCNA expression is dependent upon E2f1b, establishing a negative feedback loop [110].

Apt—Apontic (Apt), a basic leucine zipper (bZIP) TF, is involved in the development of *Drosophila* organs such as the head, heart, tracheae, nervous system and imaginal discs [112–118]. *apt* and another TF-encoding-gene *e2f1* activate expression of each other in a positive feedback loop in wing discs and salivary glands. Additionally, the genomic binding motifs of both Apt and E2f1 are clustered in the first intron of *cycE*, and Apt and E2f1 upregulate CycE expression levels in endocycling cells of the salivary gland, advancing S-phase [83]. Apt also promotes transcription of Rbf1, the *Drosophila* counterpart to the mammalian Retinoblastoma (Rb), which promotes chromatin compaction [119–121]. Rbf1 suppresses *cycE*, but only becomes active after de-phosphorylation upon S-phase initiation. Given these interactions with E2f1 and Rbf1, Apt is essential for mediating both the rapid rise and fall of CycE expression [83]. Consistent with this information, *apt* null mutation causes salivary gland cells to show less DNA content and decreased chromosome condensation. FSBP, the human homolog of Apt, regulates E2f1, CycE (CCNE1, CCNE2), and Rb1 in mammalian cells (human 293T and mouse 3T3 cells) to ensure S phase entry and chromatin condensation, suggesting the evolutionarily conserved roles of Apt [83, 118].

Snail—Snail is a zinc-finger DNA binding TF involved in establishing the mesoderm-neuroectoderm boundary during embryogenesis [122–126]. Snail is also involved in development of the prothoracic gland cells of the ring gland, as its loss or overexpression causes endoreplication cessation cell-autonomously with consequential whole organismal larval arrest [127]. In prothoracic gland cells, Snail is regulated by Target of Rapamycin (TOR) signaling, as disrupted TOR signaling reduces Snail expression but only during and prior to the critical weight check point, which coincides with assessment on nutritional status at the early third instar and determination of whether the larva commits to pupation. Some evidence suggests that Snail expression oscillates in the endocycle of the prothoracic gland cells, with high and low expression coinciding with G and S phases, respectively [127]. The mechanism of Snail regulation on the endocycle may involve transcriptional regulation of *dup* [127]. Altogether, at least in prothoracic gland cells, modulated Snail levels are important to endocycle progression.

Myb—Myb, the equivalent of MYBL2 in humans, belongs to the Myb-MuvB complex, which encompasses the dE2f2-dDp DNA-binding proteins together with the tumor suppressors Rbf1 and Rbf2. This complex is activated by Cdk1-CycA kinase, and generally suppresses endoreplication by promoting mitosis through transcriptional activation of M-phase genes, which includes Aurora B (AurB), a component of the chromosomal passenger complex [128, 129]. Knockdown of *Myb* promotes endoreplication and increases ploidy in

Drosophila S2 cells [89]. In the ovarian follicle cells, knockdown of *Myb* also increases cell ploidy and some cells acquire two nuclei, suggesting failure of cytokinesis [89]. In females expressing a null allele for Mip120, a component of the multi-protein MuvB core, ovarian nurse cells fail to undergo the mitosis-like disassembly of their polytene chromosomes at stage 5, and oogenesis is arrested between stages 7 and 8 [61].

Lov—Lov is encoded by the gene *Jim Lovell (Lov)* and contains the conserved BTB/POZ (Bric-a-Brac/Tramtrack/Broad/ Pox virus and Zinc finger) domain. Lov is involved in chromatin folding and ring canal formation [130]. During larval development, Lov is required to promote growth and endoreplication of trachea epithelial cells [131]. Absence of *lov* leads to an increased number of epithelial cells, which are consequently smaller and show loss of endopolyploidization [131, 132].

Myc—*Myc* can promote cell proliferation, cell competition and endoreplication and is conserved in vertebrates as a family of three related *Myc* proto-oncogenes [104, 105, 133, 134]. Transcription of Myc activated genes was enhanced by the ubiquitin ligase dHUWE1 (CG8184) [135]. In salivary glands, knockdown of either *Myc* or *dHUWE1* via RNAi leads to decreased nuclear size and reduced endoreplication [136]. In follicle cells, although ectopic expression of *Myc* does not induce precocious entry into the endocycle, *Myc* overexpression during the endocycle phase causes additional rounds of endoreplication and larger nuclei [85]. Myc positively regulates expression of CTP synthase, an enzyme involved in pyrimidine biosynthesis, which provides partial explanation of these phenomena [137].

2.3 Additional factors involved in polyploidization

CTPsyn—Endocycling cells are heavily reliant upon S phase replication and rapid growth, but for a round of DNA synthesis to occur, the appropriate nucleotides are required. CTP synthase (CTPsyn) is an enzyme that catalyzes UTP conversion into CTP, and it is an essential regulator of purine to pyrimidine balance in the nucleotide pool. Interestingly, CTPsyn arranges itself into a filamentous structure called the cytoophidium, a dynamic structure that reinforces proper CTPsyn activity. Immunostaining of CTPsyn in various *Drosophila* endocycling cells reliably shows the presence of large cytoophidia. Maintenance and stability of the CTPsyn filament is likely dependent on ubiquitination by the proto-oncogene Casitas B-lineage lymphoma (Cbl), an E3 ligase [137]. *Cbl* null mutants have smaller salivary gland and salivary gland cells have reduced nuclear sizes. In ovarian follicle cells, while *Cbl* heterozygous mutants do not show significant change in DNA replication, with CTPsyn knockdown, DNA replication and nuclear size is significantly reduced as compared to CTPsyn knockdown alone. Therefore, Cbl and CTPsyn interact to regulate the endocycle, possibly by maintaining balance of the nucleotide pool [137].

The chromosomal passenger complex—The Chromosomal passenger complex is a conserved hetero-tetrameric complex that regulates cell division and chromosome segregation, locating to the central spindle and centromere, respectively. The four distinct components of this complex are AurB, Incenp, Borealin-related (Borr) and the *Drosophila* Survivin ortholog Deterin (Det). Localization of each component is dependent upon the

others, and alteration of the components can result in polyploid cell formation [138]. For example, the kinase AurB is required to promote phosphorylation of Histone H3, and when AurB is depleted by RNAi in the S2 cell line, polyploidy is promoted. Chromosomal alterations such as extensive chromatin bridging at anaphase, partial chromosome condensation and abnormal chromosome segregation resulted from lagging chromatids, were also observed [139]. In ovarian follicle cells, knockdown of *aurB, borr,* or *det* resulted in large cells with polyploid nuclei. In *aurb* knockdown, some cells acquired two nuclei each with increased DNA content, indicating cytokinesis failure followed by endoreplication [89]. Regarding Mammalia, studies showed that AurB is dispensable to megakaryocyte endomitosis; however, AurB localizes to the centromere and contributes to the normal endomitotic process, making its deficiency unlikely to explain the mechanism of megakaryocyte endomitosis [140].

Dind: *diamond* (*dind*), encoding a non-conserved protein, is related to mitotic division and male meiosis [141]. In larvae with hypomorphic or null alleles of *dind*, abnormalities in chromosome morphology and number were observed in cells of the brain tissue, such as mitotic spindle disorganization, centriole fragmentation, defective chromosome segregation, chromosome rearrangements and breaks. An abnormal percentage of polyploid and hyperploid cells were also observed. Similar defects are present in *dind* mutant spermatogonia and spermatocytes. How *dind* mutants manifest this pleiotropic phenotype remains unanswered [141].

Epigenetic regulators—Epigenetic regulators such as histone methyltransferases control gene expression by modifying the condensation state of chromatin. Histone modifications are required for control of DNA replication. In salivary gland polytene chromosomes, latest replicating regions are often characterized by pericentric heterochromatin with methylation enriched on histone H3 Lysine K9 (H3K9) residues [142]. These regions are often left unreplicated during endoreplication, which results in decreased ploidy in this region. When the histone residue H3K9 residue is mutated through histone gene replacement strategy (*H3K9R*), DNA copy number increases in under-replicated regions of the salivary gland, indicating that under-replication in the latest replicating regions is dependent on H3K9 methylation [142].

Additionally, under-replicated regions of salivary gland polyploid cells are controlled by Suppressor of Under-Replication (SuUR), a chromatin binding protein that associates with H1 to establish proper underreplication. Interestingly, during the S phase of the endocycle, H1 is located in the late replicating regions and gets redistributed as endoreplication proceeds, which increases DNA copy number in the late replicating regions [8].

The Lysine demethylase 5 (KDM5) is a histone demethylase involved in chromatin-mediated regulation of transcription, and promotes endoreplication in prothoracic gland cells. KDM5 promotes endoreplication by activating the expression of the receptor tyrosine kinase Torso, which results in the activation of MAPK signaling pathway in prothoracic gland cells [143]. Hat-trick (htk), a chromodomain protein, is involved in chromatin remodeling and mostly expressed in the oocyte nucleus. When *htk* is null mutated in

ovarioles, egg chambers display extranumerary nurse cell nuclei with decreased ploidy, as evidenced by decreased nuclear size [144].

LINC Complex—The Linker of Nucleoskeleton and Cytoskeleton (LINC) connects the cytoskeleton to the nuclear lamina and allows the transmission of mechanical inputs from the cytoplasm to nuclear membrane components. Null mutation of LINC components *klarsicht* (*klar*) and *klaroid* (*koi*) increases DNA content in myonuclei, disrupts cell cycle progression, and promotes recurring endoreplication [145]. Knockdown of β-PS-integrin distorts adhesion between tendons and muscle fibers, which also increases DNA content in myonuclei, suggesting that increased DNA content in LINC mutant myonuclei is caused by changes in nuclear mechanotransduction [145]. Barrier-to-autointegration factor (BAF) bridges the nuclear lamina to chromatin, and possibly acts downstream to LINC complex-induced nuclear mechanotransduction, leading to chromatin remodeling and changes in cell cycle gene expression [145].

2.4 Signaling pathways involved in the endocycle

Cells use established signaling pathways to communicate information, such that specific signals can produce predictable and reproducible results within a target tissue. Signaling pathways are vital in the orchestration of tissue development and cell differentiation, including those select cells that enter the endocycle. In *Drosophila*, a wide variety of cell types programmed for polyploidy are affected by cell signaling. Additionally, transgenes and mutant genes can perturb a variety of signaling pathways and cause endoreplication in a population of cells that would have otherwise remained diploid. This section will focus on pathways that have been shown to be involved in regulating the endocycle, including Notch, Insulin/Insulin-like growth factor (IIS) and target of rapamycin (TOR), Hippo, Ras/MAPK and c-Jun N-terminal kinase (JNK).

The Notch Pathway—The Notch signaling pathway is a conserved pathway involved in cell fate determination, differentiation, proliferation and apoptosis [146]. For example, mammalian Notch has been identified as a positive regulator of megakaryocyte specification but inhibits megakaryocyte maturation [147, 148]. In *Drosophila*, Notch signaling starts by interaction between the Notch receptor and ligands Delta and Serrate found on the surface of neighboring cells. Upon receptor activation by ligands Delta and Serrate, ADAM metalloprotease and γ secretase cleave the Notch receptor between the transmembrane and intracellular domains, releasing the Notch intracellular domain (NICD) into the cytoplasm. NICD is translocated through endosomal vesicles to the nucleus where it forms a complex with DNA binding proteins, Suppressor of Hairless (Su(H)) and Mastermind (Mam). Ultimately, this complex drives the transcription of target genes involved in various cellular processes.

In ovarian follicle cells, Notch signaling triggers differentiation of immature diploid cells to become polyploid by initiating the mitotic cycle to endocycle transition (M/E switch). Activation of the M/E switch takes place during stages 6 and 7 of oogenesis. The germline cells (nurse cells and the oocyte) upregulate the Delta ligand to activate the Notch receptor in the follicle cells, which in turn promotes upregulation of the zinc finger

TF Hindsight (Hnt) and downregulation of homeobox TF Cut. Repression of Cut leads to upregulation of Fzr, which enables endocycle entry. Furthermore, it has been shown that Notch downregulates Dap, the Cdk2 inhibitor, and String (Stg), a positive regulator of Cdk1, a mitotic CDK. Due to these changes induced by Notch signaling, follicle cells undergo three rounds of endoreplication to become polyploid [56, 57, 60, 149, 150].

Development of subperineurial glia (SPG) illustrates another example of Notch-regulated ploidy and variant cell cycle determination. SPG cells acquire polyploidy through endocycle and endomitosis. Disrupting Notch signaling increases the ratio of multinuclear to mononuclear SPGs, suggesting that Notch in SPGs is involved in maintaining endocycle and inhibiting endomitosis [151].

The IIS and TOR Pathways—The Insulin/Insulin-like growth factor (IIS) and TOR signaling pathways are conserved pathways that work together to control cellular responses to nutritional stimuli [152]. Regarding dietary restrictions, the inhibitory regulation of IIS and TOR signaling on progression of the endocycle are present across many tissues, including the larval fat body, gut and proventriculus, and salivary gland, where translation of E2F1 has been demonstrated to be impacted via IIS and TOR signaling in response to changing nutrition [79, 153].

In the ovary, ISS and TOR signaling have the potential to alter cellular response to Notch signaling if nutrient availability is poor and are thus directly relevant to follicle cells that are programmed to undergo the M/E switch. Mechanistically, starvation triggers a decrease in insulin-like peptide within the follicle cells, and when Insulin-like receptor (InR) is unbound to its insulin-like peptide, the TF Forkhead box (FoxO) is translocated to the nucleus, where it activates Cut expression, creating a regulatory loop on Notch and pausing activation of the M/E switch. This M/E switch standstill is reversible upon refeeding the flies, thereby causing the follicle cells to enter the endocycle [154]. In the midgut, *InR* activity is required to promote enterocyte differentiation. Intestinal stem cell clones with null mutations of *Pi3K, TOR,* or *InR* are arrested in a diploid state, while overexpression of *InR* or *Rheb* in enteroblasts produces enlarged cells with increased ploidy [155].

The Hippo Pathway—The Hippo pathway, first identified in *Drosophila*, is a conserved pathway in metazoans, and is involved in the control of tissue growth and organ size [156]. The core of this pathway encompasses four components: the Hippo (Hpo), Salvador (Sav), Warts (Wts) and Mob-as-tumor-suppressor (Mats), which collectively form the Hippo kinase complex [157]. This complex negatively regulates the nuclear translocation of Yorkie (Yki), which is the downstream effector of the Hippo signaling cascade that drives the expression of genes related to growth/proliferation such as *Myc, ban, diap1, E2f1,* and *cyclins A, B* and *E* [157].

Yki and its cofactor Multiple Ankyrin repeats Single KH domain (Mask) regulate polyploidy in the sub perineurial glia cells by participating in a double-negative feedback loop with the microRNA miR-285. Mechanistically, miR-285 suppresses Yki activity by direct contact with Mask, resulting in downregulated expression of Yki's target CycE. miR-285 levels are increased by downregulation of *yki* or *mask*, and decreased by *yki* overexpression.

This double-negative feedback is essential to assure proper activity of these components and regulate polyploidy in sub perineurial glia cells. Disturbance of this double-negative feedback loop increases ploidy levels and nuclear sizes, as evidenced by *miR-285* knockout or *yki* overexpression [158].

Upon wounding, loss of tissue can be replaced by cell enlargement through endoreplication. Yki regulates endoreplication in the injured fly abdomen after wounding, and knockdown of *yki* disrupts wound response [159, 160].

In Mammalia, Hippo has been reported to suppress tumorigenesis and cell ploidy through Skp2 [161]. Tetraploidy caused by cytokinesis failure can activate the Hippo tumor suppressor pathway [162].

The RAS/MAPK Pathway—*Drosophila* Ras shows substantial amino acid sequence homology (75%) to its mammalian counterpart [163, 164], and participates in the initiation of the MAP kinase cascade, leading to the activation of RAF, MEK and ERK (MAPK), respectively. As the final kinase, ERK translocates to the nucleus where it activates various TFs involved in cell growth and proliferation.

Ras proteins are small GTPases that switch from active to inactive states upon GTP to GDP binding [165]. The GTPase enzymatic domain of Ras is a frequent target for oncogenic mutations because loss of this enzymatic activity confers constitutive activation upon Ras [166]. Generally, knockdown of Ras stalls cells at G1, while activated Ras promotes the G1/S transition in both the cell cycle and endocycle by upregulating CycE post-transcriptionally [167, 168]. Additionally, Ras shortens G1 by upregulating Myc [163]. Interestingly, in the salivary gland, the endocycle can be affected indirectly by ectopic localization of Ras upon knockdown of Rab11, a vesicle trafficking GTPase. These salivary gland cells show reduced DNA content and nuclei size along with decreased transcription levels of the endoreplication regulators CycE, E2f1 and Gem [169].

In *Drosophila,* oncogenic Ras (Ras^{V12}) combined with disruption of the apico-basal polarity gene *scribble* (*scrib*) leads to tumor formation in the wing disc. These events are associated with the activation of Yki and JNK pathways, which are known to downregulate CycB, leading to polyploid cell formations in these wing disc tumors [170]. In the pylorous, Ras^{V12} expands the pylorous by cell proliferation in the larval stage but endoreplication in the adult [92].

The JNK Pathway—The JNKs is a conserved signaling pathway involved in cell death, immunity and DNA damage [171]. JNK signaling is activated under cell stress or loss of cell polarity. Upon activation, tumor necrosis factor (TNF) Eiger binds to its receptors Wengen or Grindelwald, and these receptors activate the core kinase cascade that eventually terminates in the final JNK called Basket (Bsk), which activates via phosphorylation various TFs, which upregulate genes often related to caspase-mediated apoptosis, including *head involution defective* (*hid*) and *reaper* (*rpr*) [172].

JNK signaling has a dual role in polyploid cell development and tumorigenesis that depends on the genetic context of the cell population and crosstalk with other signaling

pathways. In imaginal epithelia with *peanut* knockdown, JNK acts as a tumor suppressor by postranscriptionally suppressing the apoptotic-inhibitor Diap1 in the cells having undergone cytokinesis failure, promoting apoptosis of the tetraploid cells. Additionally, JNK posttranscriptionally represses the M-phase inducer String (Cdc25 homolog), preventing polyploid, error-prone mitosis. Interestingly, these tumor-suppressive effects can be overcome with Yki overexpression, which promotes the transcription of M-phase genes [173]. Notwithstanding the role JNK as a tumor suppressor, JNK signaling can also promote giant polyploid cells via endoreplication in certain neoplastic overgrowths. Some of these tumors include those produced by loss of an endocytic gene *rab5*, *vps25*, *erupted*, or *avalanche*, and epithelial malignant tumors produced by $Ras^{V12}/scrib^{-/-}$ or $Ras^{V12}/dlg1^{-/-}$ mutants. Mechanistically, endoreplication is promoted through downrugulation of CycB by JNK signaling and cooperation by Yki causes upregulation of Diap1, preventing cell death [170].

4. Polyploidy in wound repair and tissue homeostasis

4.1 Wound-induced polyploidy

During organismal development, cells are exposed to stress conditions that can result in tissue damage and consequential cell loss. To compensate for the empty space left by cell loss, neighboring surviving cells with limited division capacity can undergo polyploidization to enlarge cell size. This mechanism is called wound-induced polyploidy (WIP) [174], and is conserved between *Drosophila* and mammals. For example, in mammalian organs with limited division potential such as the heart, liver, cornea, kidney and bladder polyploidization occurs in response to tissue injury [175–179].

WIP can be induced by puncturing the fly abdomen. Quiescent epidermal cells surrounding the puncture wound re-enter S phase and become polyploid. Epidermal cells surrounding the wound undergo WIP via activation of Yki, a downstream effector of the Hippo pathway. Yki activates endocycle genes (*Myc* and *E2f1*) and the microRNA *bantam (ban)*. In contrast, AP-1 activation by JNK restricts polyploidization and Yki activation [160]. Interestingly, epithelial cells with *stg* or fzr^{RNAi} expression that use mitosis as a repair mechanism in lieu of WIP are more prone to accumulate DNA damage and mitotic errors, demonstrating the advantage of WIP as a repair strategy in epithelial tissue [180].

The *Drosophila* intestine has also been used to study WIP. Though injury to the hindgut pylorus is repaired by mitosis during the larva stage, endoreplication is the preferred repair mechanism during the adult phase. Endoreplication in the adult pylorus is regulated by Fzr, a component of APC/C, which helps with the degradation of mitotic cyclins such as CycA and CycB [92]. The intestinal midgut also demonstrates WIP. Upon damage caused by the enteropathogen *Pseudomonas entomophila*, midgut stem cells transform into enteroblasts and enterocytes. This transformation is achieved through compensatory endoreplication via EGFR/MAP kinase signaling and results in enterocytes with higher ploidy levels [155].

4.2 Compensatory cellular hypertrophy in tissue homeostasis

Tissue homeostasis corresponds to maintenance of tissue integrity and size upon adverse conditions such as the appearance of mutations that lead to the formation of aberrant cells that are slower-growing or structurally defective. In *Drosophila*, slower-growing cells can be obtained by loss of the TF *Myc* or the protooncogene *Ras*, while structurally defective cells can be induced by loss of apicobasal cell polarity, which can be caused by mutations in genes like *discs large* (*dlg*), *scribble* (*scrib*) and *lethal giant larvae* (*lgl*). Maintaining homeostasis amidst these mutant cells, cell competition is a process used by tissues where less fit cells called the “losers” are eliminated by surrounding neighboring cells, the “winners”. In mitotic epithelia, eliminated loser cells are compensated by neighboring cell proliferation, “compensatory proliferation”. While in post-mitotic tissues, such as endocycling follicle cells, the winner cells perform compensatory cellular hypertrophy, a process in which the winner cells increase their nuclei size two to four times by undergoing extra rounds of endoreplication [104, 181–183].

5. Polyploid mitosis and genomic instability

Genomic instability refers to loss of genome integrity and is characterized by frequent mutations, including chromosomal rearrangements and aneuploidy. Research on genomic instability is of therapeutic value, given that genomic instability is a hallmark of cancer [184, 185]. In *Drosophila*, tumorigenesis is similarly associated with genomic instability; for example, by disrupting chromosome segregation to promote genomic instability in imaginal disc epithelia, many features of human tumors are recapitulated, including loss of apical-basal polarity, cell delamination, basement membrane degradation, and tumor invasiveness. Substantial evidence from various tissues within the *Drosophila* model suggests error-prone, polyploid mitosis as a source of genomic instability [48, 90, 186].

Rectal papillae cells can be used to study polyploid mitosis because mitotic division is programmed to follow endoreplication in rectal papillae at metamorphosis. The switch from endoreplicative to mitotic division compromises the genomic stability of rectal papillae, with instability indicated by the presence of broken and acentric chromosomes, lagging chromosomes, and chromatin bridges during an elongated anaphase [48]. Chromosomal abnormalities are also observed in mitotic polyploid ileum cells of the mosquito *Culex pipiens*, supporting the rationale that genomic instability is due to an inherent imprecision of polyploid mitosis instead of a singular physiological property of *Drosophila* rectal papillae [48]. Interestingly, rectal papillae accumulate centrioles during the endocycle stages, leading to centrosome amplification and multipolar division at post-endocycle stages. The error-prone nature of multi-polar division causes a high-frequency of aneuploidy [186]. Consequently, rectal papillae possess multiple mechanisms to tolerate and minimize the effects of chromosomal imbalances due to mitotic infidelity. First, unlike the canonical DNA damage response, p53 is not activated to mediate cell cycle arrest or apoptosis in the presence of double-strand breaks in rectal papillae [186]. Tolerance to DNA damage is not unusual for normal development, as many endocycling cells in both flies and humans will also silence cell death genes [15, 187]. Second, rectal papillae activate Blm helicase and Fanconi anemia proteins to coordinate proper alignment and segregation of broken

chromosomes and acentric fragments to the poles, helping to prevent micronuclei formation at post-endocycle mitosis [188]. Cytoplasmic sharing has also been investigated as a means whereby specialized gap junctions neutralize the genomic imbalances between nuclei [189].

In contrast to rectal papillae, in most other organs, endoreplication is followed by senescence, but manipulation of the developmental program of the ovary has allowed research on post-endocycle mitosis in ovarian follicle cells, which normally undergo the M/E switch at stages 6 and 7 in a Notch dependent manner [56, 60, 149, 150]. Independent of Notch signaling, cells with *CycA* knockdown or *fzr* overexpression can enter precocious endocycles. In either case, endocycle induction protects against apoptosis caused by ionizing gamma radiation [90]. Given relief from heat shock-induced *fzr* overexpression, cells having undergone precocious endocycles re-enter mitosis – and these cells regain susceptibility to radiation-induced apoptosis. Post-endocycle mitosis in these cells is error-prone, which was evidenced by chromosomal loss and fragmentation, and centrosome amplification [90].

Centrosome amplification has been linked to spindle assembly defects and tumor growth and formation [190]. Sak kinase (PLK4 in humans) is required for centriole duplication, and when overexpressed in larval neuroblasts, centrosome amplification occurs. Neuroblasts showing amplified centrosomes initiate multipolar spindle assembly, but this aberration is frequently resolved at the spindle assembly checkpoint, with only a slight delay as the supernumerary centrosomes cluster together, by which point spindle bipolarity is re-established. Regardless, *sak* overexpression neuroblasts show subtle differences of spindle alignment in reference to cortical cues. To determine if centrosome amplification can contribute to tumorigenesis, the Sak kinase overexpressed brain tissue was transplanted into wild type hosts. Remarkably, up to 20% of the hosts developed tumors, and several of these tumors metastasized to locations distant from the injection site [74].

Research using the *Drosophila* model demonstrates several interesting phenomena. First, polyploid cells can become mitotic. This post-endocycle mitosis can lead to genomic instability. Polyploid mitosis can be characterized by aneuploidy and other chromosomal defects. In some but certainly not all contexts, error-prone mitosis caused by extranumerary centrosomes contributes to tumorigenesis; this mechanism is also demonstrated in human cancers [191]. Future investigations on the causal relation between polyploid mitosis and genomic instability, the characterization of multipolar mitosis, as well as the variability by which genomic instability is tolerated and processed by cells sheds light on the basics of cancer biology.

6. Polyploidy in *Drosophila* tumor models

Polyploid cells can be found in human cancer samples as PGCCs, which are associated with metastasis and anticancer therapy resistance since PGCCs are a source of genomic instability and aneuploidy [22, 23, 192–194]. Moreover, using genomic sequencing of tumor biopsies, the presence of polyploidy has been shown to shape prognosis and progression of advanced cancers [24, 27] In *Drosophila,* evidence confirms that polyploid cells play important roles in tumorigenesis, tumor growth and invasiveness [133, 170, 173]. This section will address some processes of polyploid cell formation in *Drosophila* tumors, while

elaborating upon the relation and contributions of polyploidy to tumorigenesis and tumor growth.

In the *Drosophila* eye-antennal disc, activated Ras (Ras^{V12}) and simultaneous loss of *scrib* leads to the formation of tumors with the presence of polyploid cells [170]. Similarly, in the wing and eye discs, polyploid cells can be obtained by loss-of-function mutations in endocytic "neoplastic tumor suppressor" genes *rab5*, *avalanche*, *erupted* or *vps25*. In $rab5^{-/-}$ and $Ras^{V12}/scrib^{-/-}$ mutants, endoreplication is caused by synergic activation of Yki that activates Diap1 and cooperates with JNK to cause downregulation of CycB. In $Ras^{V12}/scrib^{-/-}$ tumors, tumor growth is suppressed by eradication of polyploid cells through repression of endoreplication via co-overexpression of CycB or by overexpression of the dominant negative form of the *Drosophila* JNK, Basket. In addition, co-overexpression of CycB inhibited $Ras^{V12}/scrib^{-/-}$ tumor metastatic invasion [170]. Thus, polyploid cells in $Ras^{V12}/scrib^{-/-}$ tumors are suggested as an essential factor for tumor growth and metastasis.

A *Drosophila* salivary gland imaginal ring (ImR) tumor model provides further demonstration of the role of polyploid cells in the tumor initiation and progression [195, 196]. The ImR tumor is formed in a transition zone (TZ) region located between the diploid ImR cells and polyploid salivary gland cells. The TZ region is rich in JNK and Janus kinase (JAK)-signal transducer and activator of transcription (STAT) activity, making this region a tumor hotspot. Because of high activity of JAK-STAT and JNK signaling, Notch induction is sufficient for tumor formation in this region [195]. In this tumor, in addition to polyploid cells re-entering mitosis, continued endoreplication and depolyploidization also contribute to tumor progression. These multiple cell cycle mechanisms ensure tumors always contain a proportion of polyploid cells during tumorigenesis, indicating a role of polyploid cells during tumor progression. Polyploid cell division in the ImR tumor is error-prone since multiple centrosomes, chromosome bridges, lagging chromosomes and asymmetric division were frequently observed, leading to tumors with high chromosome instability. Finally, RNA-seq analysis of ImR tumors showed upregulation of several DNA damage response and repair genes that are also active during meiosis. Knockdown the upregulated DNA damage response and repair genes resulted in depolyploidization defects in the ImR tumors, suggesting a relevant role of DNA damage response and repair pathway during tumor formation [196]. Ploidy reduction also contributes to tumor formation in mammals [26]. Proliferating polyploid cells in liver tissue produce aneuploidy, and increasing ploidy dynamics in liver tissue can raise the risk of liver cancer [197–199].

Tumorigenesis and tumor growth are dependent on cell competition in epithelial cells overexpressing epidermal growth factor receptor (EGFR) combined with microRNA 8 (miR-8) [133]. Interestingly, miR-8 downregulates Peanut, a Septin family protein required for cytokinesis, promoting tumor formation and metastasis when EGFR is overexpressed; however, depletion of Peanut with EGFR overexpression does not lead to tumor formation, raising the possibility that miR-8 has other targets besides Peanut [133]. These epithelial tumors showed metastatic potential and featured giant cells with enlarged nuclei and banding patterns typical of polytene chromosomes. Characteristic to a super-competitor behavior, the giant tumor cells showed elevated Myc levels and induced apoptosis and engulfment of neighboring wild type cells. Using the MARCM method, when *Myc* was

overexpressed in neighboring cells, EGFR + miR-8 clones did not produce giant cells or induce neoplasia. Additionally, reducing *Draper* (which is involved in engulfment) in tumor cells inhibited cell enlargement and tumorigenesis in EGFR + miR-8 clones. Furthermore, suppressing apoptosis in the EGFR + miR-8 tissue by expression of p35 or Diap-1 also inhibited tumor formation. Taken together, cell competition mediated through induction of apoptosis and engulfment are suggested to be required for tumor formation in this tumor model [133].

Depletion of *peanut* in the wing disc epithelium leads to cell cytokinesis failure, resulting in tetraploidy and apoptosis. Apoptosis upon cytokinesis failure is mediated through JNK signaling, which suppresses the apoptosis inhibitor Diap1 [173]. Epithelial wing disc tumors are induced when *peanut* depletion is combined with either Yki overexpression or Ras^{V12}. Tumors driven by Yki overexpression and peanut depletion cause cells to demonstrate putative malignant features such as cell and nuclear enlargement, increased centrosome count, increased *matrix metalloproteinase 1* (*Mmp1*) enzyme, invasive potential and polarity disruption. Yki overexpression in these tumors limits cell death through Diap1 expression and through upregulation of Stg which stimulates progression of the cell cycle. Concomitantly, JNK limits Stg expression, demonstrating a tumor suppressive role that is overcome by Yki overexpression [173].

Drosophila tumor models share aspects existent in human solid tumors such as the presence of genomic instability and polyploid cells [23, 192–194]. These Tumor models improve biological understanding of the various factors and mechanisms that involve polyploid cells in tumor formation. These tumor models implicate polyploid cells as key drivers of tumor initiation and growth and dispute the notion that polyploid cells are merely consequential to tumorigenesis.

Conclusion

Polyploid cells are prevalent during *Drosophila* development, with the functional aspects of polyploidy being multifold and unique across organs. In tissues where mitotic division is no longer an option, polyploidization serves as a response against tissue integrity loss and is inherent to wound repair and tissue homeostasis. The programs leading to polyploidy are subject to a myriad of regulatory factors, providing cell types with endocycles of diverse variation. For example, comparing the ovary and salivary gland, Dap oscillates at different concentrations, possibly driving differential under-replication of euchromatic regions [87]. Nevertheless, some common themes on all endocycles exist. First, cells downregulate mitotic cyclins or decrease mitotic CDK activity, and second, regulated Cdk2-CycE activity and CycE oscillation is necessary to appropriately alternating G and S phases. Furthermore, the endocycle can be affected by internal growth restraints and is responsive to cellular signaling.

The contributions of polyploidy to tumorigenesis and tumor progression have only started to be elucidated. Mounting evidence indicates error-prone, polyploid mitosis as a source of genomic instability, which in various and certain contexts can drive tumor formation and malignancy. Fruit fly research will shed light on the interaction of signaling pathways

and various factors involved in the formation of tumorigenic polyploid cells. Rather than having a passive role, in various tumor models, polyploid tumor cells are implicated as key instigators in the progression of tumorigenesis and tumor growth. Considering the wide occurrence of PGCCs in human solid tumors, studies using the *Drosophila* model that characterize unique facets of polyploid cells are of therapeutic significance.

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Glossary

C ploidy values
DNA content of a cell

N ploidy values
the number of chromosome sets

Polyploidization
process of acquiring polyploidy

Haploidy
one complete set of chromosomes

Diploidy
two complete haploid sets of chromosomes

Hypserploidy
chromosome number slightly greater than an exact multiple of the haploid ploidy value

Polyploidy
ploidy is greater than two

Endopolyploidy
polyploidy via endoreplication

Aneuploidy
a difference of chromosomes within a haploid set

Polytene chromosome
fusion of multiple chromosomes showing characteristic banding patterns

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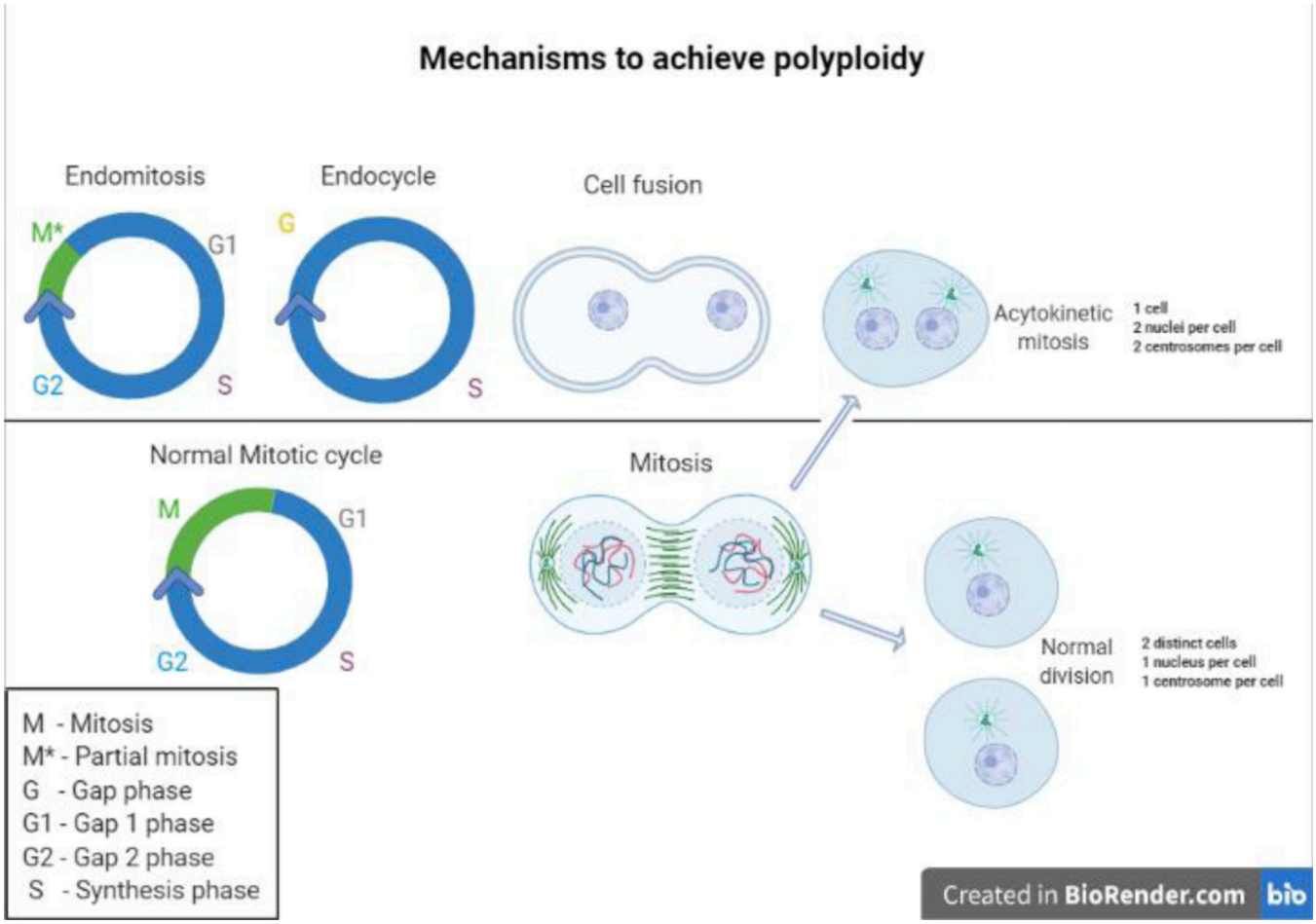

Figure 1.

Endomitosis encompasses G1, S, G2 and partial M phase without karyokinesis and cytokinesis. Endocycle comprises the G and S phase. In cell fusion, cells merge, resulting in a multinucleated cell. The mitotic cycle includes G1, S, G2 and M phase and ends with two single nucleated cells.

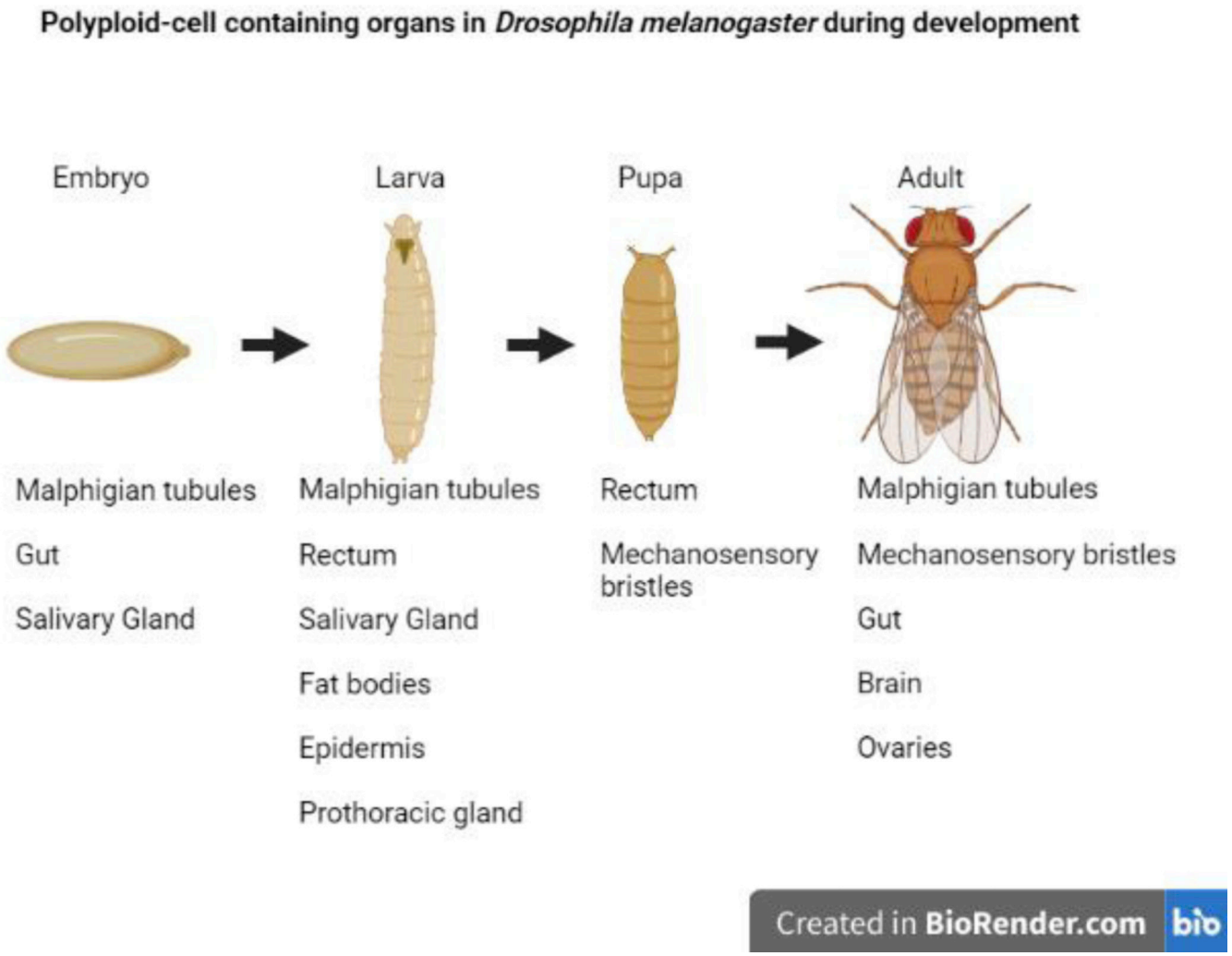

Figure 2.
Through different *Drosophila* life cycle stages, polyploid cells promote development and growth in distinct organs.

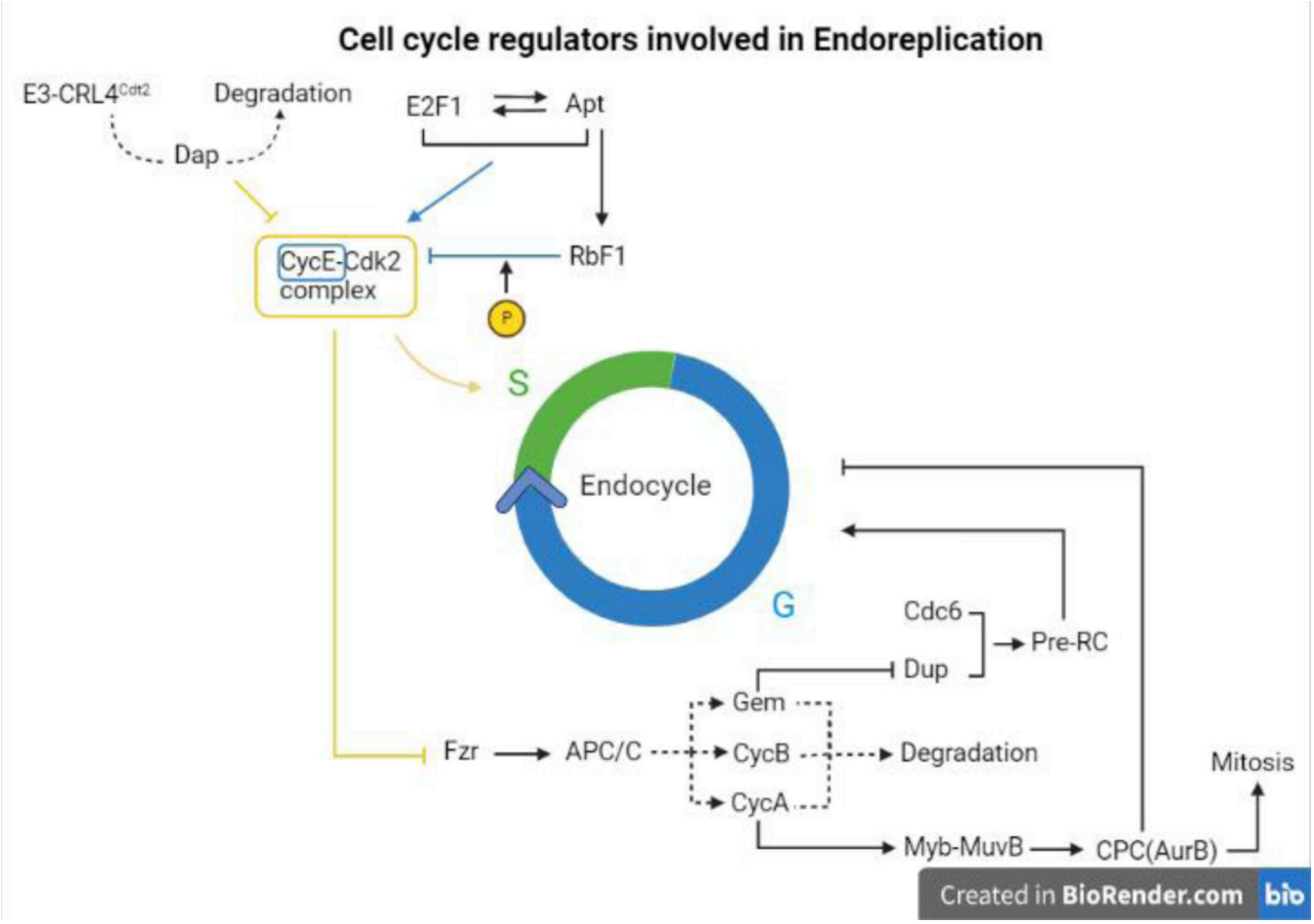

Figure 3.

Depiction of the endocycle process. Specific expression of cell cycle regulators promotes S and G phase and evasion of M phase.

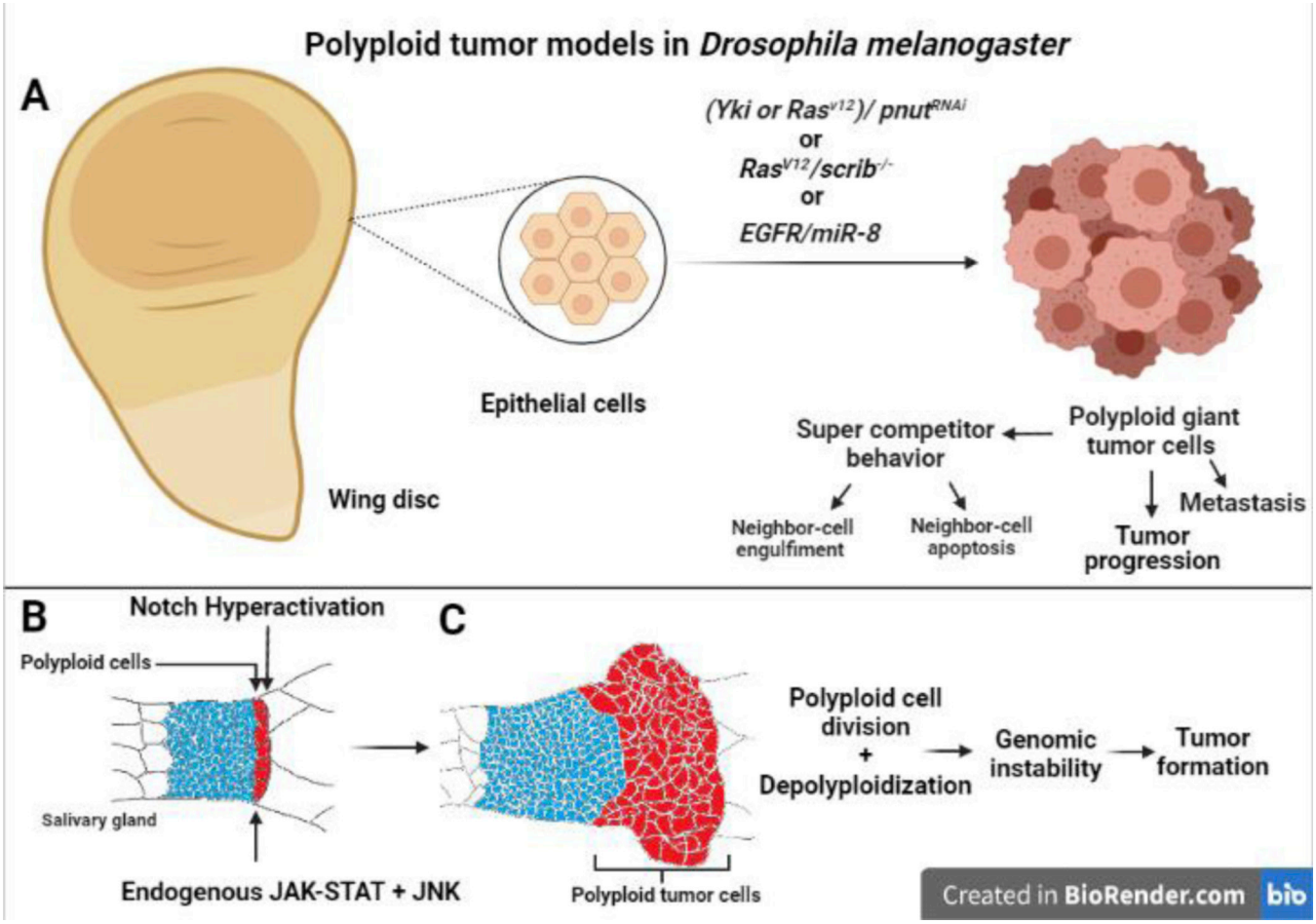

Figure 4.

Different tumor models in *Drosophila* containing polyploid-tumor cells. (A) Ectopic expression of oncogenes in combination with tumor suppressor inhibition transforms the wing-disc epithelia into neoplasms containing polyploid giant tumor cells with features that recapitulates certain hallmarks of cancer. (B) Notch hyperactivation promotes neoplastic tumorigenesis in imaginal ring polyploid cells. (C) Polyploid-tumor cells re-enter mitosis and depolyploidization resulting in chromosomal instability.