



Regulatory Mechanisms of Cell Polyploidy in Insects

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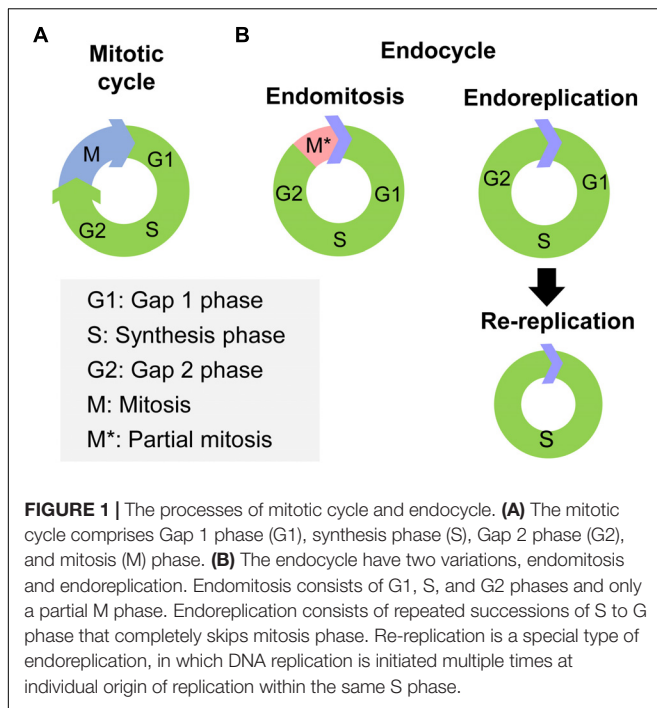
Polyploidy cells undergo the endocycle to generate DNA amplification without cell division and have important biological functions in growth, development, reproduction, immune response, nutrient support, and conferring resistance to DNA damage in animals. In this paper, we have specially summarized current research progresses in the regulatory mechanisms of cell polyploidy in insects. First, insect hormones including juvenile hormone and 20-hydroxyecdysone regulate the endocycle of variant cells in diverse insect species. Second, cells skip mitotic division in response to developmental programming and conditional stimuli such as wound healing, regeneration, and aging. Third, the reported regulatory pathways of mitotic to endocycle switch (MES), including Notch, Hippo, and JNK signaling pathways, are summarized and constructed into genetic network. Thus, we think that the studies in crosstalk of hormones and their effects on canonical pathways will shed light on the mechanism of cell polyploidy and elucidate the evolutionary adaptations of MES through diverse insect species.

Keywords: endocycle, juvenile hormone, 20-hydroxyecdysone, mitotic/endocycle switch, cell cycle

INTRODUCTION

Cell polyploidy generated by endocycle is a cell cycle variant that undergoes multiple rounds of nuclear genome duplication in the absence of cell division (Zielke et al., 2013). In canonical mitotic cell cycles, cells pass through Gap 1 phase (G1), synthesis phase (S), Gap 2 phase (G2) and end in mitosis (M), thus the genetic materials of the mother cell are duplicated and delivered to two daughter cells (**Figure 1A**). However, in endocycles, cells increase their genomic DNA content without cell division (Lee et al., 2009). Two variations of endocycle are endoreplication and endomitosis (Zielke et al., 2013). Endoreplication consists of successive Synthesis phase to Gap phase that completely skips mitosis (Lee et al., 2009; Maldonado et al., 2019; **Figure 1B**). Cells that undergoes endoreplication result in a single, enlarged, polyploid nucleus (Calvi, 2013). A special type of endoreplication is re-replication, in which DNA is initiated multiple times at individual origins of replication within a single S phase, provoking site-specific replication of a unique sequence (Lee et al., 2009; **Figure 1B**). Thus highly amplified and underreplicated DNA sequences at different loci form supersized multifiber-like chromosomes called polytene chromosomes (Stormo and Fox, 2017). While, endomitosis consists of G1, S, G2, and partial M phases, which produces cells with a single giant nucleus, which may subsequently fragment into a multinuclear appearance (Zielke et al., 2013; **Figure 1B**).

The biological purposes of endocycle include tissue growth, blood-brain barrier formation, immune response, nutrient support, and conferring resistance to DNA damage based on tiss



types and developmental stages (Lilly and Spradling, 1996; Zhang et al., 2014; Lynch and Marinov, 2015; Bretscher and Fox, 2016; Wu et al., 2018; Zülbahar et al., 2018; Maldonado et al., 2019). Endocycle is considered a low-cost strategy to increase the cell and/or tissue size and efficiently produce massive molecules needed for specific functions, which avoids spending more time, materials, and energy to go through complete mitotic cycles (Edgar and Orr-Weaver, 2001; Lynch and Marinov, 2015; Maldonado et al., 2019). Thus, polyploidy cells have advantages in the adaptation of organisms to environment; however, they can also have detrimental effects on fertility and fitness owing to genomic instability, mitotic and meiotic abnormalities, and gene expression and epigenetic changes (Zhimulev, 1996; Van de Peer et al., 2017). On the other hand, unpredicted cell polyploidy is closely related to the development and progression of cancer in mammals (Storchova and Pellman, 2004; Davoli and de Lange, 2011; Fox and Duronio, 2013; Coward and Harding, 2014). Clinically, endocycle and polyploidy have been observed in cancerous tissues, with their occurrence ranging from 11% in stomach carcinoma to 54% in liver adenocarcinoma (Storchova and Pellman, 2004; Shu et al., 2018). Therefore, these findings provided important clues for revealing the essential roles of polyploidy in normal development and tissue, homeostasis, as well as the relationship between polyploidy and the progression of cancer in vertebrate animals.

Previously, some scientists have summarized the key genes that play a role in cell polyploidy and mainly focus on the cell division arrest. The interactive regulation of E2f1, cyclin E, and cyclin-dependent kinase (CDK) promote endocycle occurrence through bypassing many of the processes of mitosis in insects and mammals (Zielke et al., 2013). And the biological purposes of cell polyploidy in many different organisms are

generally discussed (Edgar and Orr-Weaver, 2001; Lee et al., 2009; Fox and Duronio, 2013). Insects as the biggest groups in invertebrates have common phenomena of cell polyploidy; however, a thorough summary of previous studies involving in more extensive insect species is lacking except in *Drosophila*. Here we review the cells types with polyploidy and recent progresses in the regulatory mechanisms of cell polyploidy in insects with special emphasis on the aspects of hormones action and environmental stimuli. In addition, we constructed a regulatory network based on previous and recent reported signaling pathways involved in MES. Finally, we proposed a promising MES study system and potential directions in cell polyploidy study in insects.

POLYPLOID CELLS IN INSECTS

Polyploidy is commonly observed in highly metabolic tissues or cells in both complete and incomplete metamorphosis insects (Table 1). The fat body, midgut, muscle, Malpighian tubules, and nurse cells are the most reported tissues or cells with polyploidy (Zhimulev, 1996; Lee et al., 2009; Wu et al., 2018; Maldonado et al., 2019). The ploidy levels ranged from 4C in the fat body cells of *Locusta migratoria* to 2048C in the salivary glands of *Drosophila* and varied from 4C to 64C in different cells of single tissue (Hammond and Laird, 1985; Guo et al., 2014). Most of these polyploid tissues or cells can rapidly and massively synthesize proteins upon necessity during development, reproduction, immunity, flight, and other life activities (Ray et al., 2009; Fox and Duronio, 2013; Rangel et al., 2015; Wu et al., 2016).

In the fruit fly *Drosophila melanogaster*, larval growth is achieved primarily via endoreplication (Edgar and Nijhout, 2004). Most *Drosophila* larval tissues are composed mainly of polyploid cells including the salivary glands, fat body, germline cells, subperineurial glia, epidermis, gut, trachea, and Malpighian or renal tubules (Lee et al., 2009). The giant salivary gland cells undergo about 10 endocycles, resulting in polytene chromosomes (Hammond and Laird, 1985; Maldonado et al., 2019). In adult *Drosophila*, the maximum polyteny level is 64C in the midgut and 256C in Malpighian tubules, respectively (Lamb, 1982). *Drosophila* papillar cells become polyploid and naturally accumulate broken acentric chromosomes but do not apoptose/arrest the cell cycle, thus they can divide and survive despite high levels of DNA breakage (Zhang et al., 2014; Bretscher and Fox, 2016). The fat body generates polytenic cells through re-replication (Juhász and Sass, 2005). In the female germline, nurse cells become polyploid during oogenesis, enabling them to provide vast amounts of maternal messages and products for the developing oocyte, whereas the somatic follicle cells that surround the egg undergo only three endocycles from stages 7–9 to reach a ploidy level of 16C, which facilitates the high levels of functional gene expression needed for reproduction (Calvi and Spradling, 1999; Royzman et al., 2002). Interestingly, the subperineurial glia expand enormously and become polyploid undergo both endoreplication and endomitosis, allowing it to accommodate

TABLE 1 | Tissues or cells with polyploidy in insects.

Order	Species	Tissue/cell type	Function	References
Diptera	<i>Drosophila melanogaster</i>	Brain/subperineurial glial cells	Blood-brain barrier	Unhavaithaya and Orr-Weaver, 2012; Zülbahar et al., 2018
		Ovary/nurse cells	Provide vast amounts of maternal messages and products for the developing oocyte	Royzman et al., 2002
		Ovary/follicle cells	Oocyte maturation and egg chamber development/eggshell gene amplification, DNA damage resistance	Calvi and Spradling, 1999
		Papillar cells	Repress the apoptotic response to DNA damage	Zhang et al., 2014; Bretscher and Fox, 2016
		Salivary glands	Synthesis and secretion glue proteins	Hammond and Laird, 1985; Buntrock et al., 2012
		Fat body	–	Juhasz and Sass, 2005
		Hindgut/rectal papillae	–	Fox and Duronio, 2013
		Trachea	–	Fox and Duronio, 2013
		Malpighian tubules	Absorb water, solutes, and wastes and excrete them as nitrogenous compounds	Lamb, 1982
		Renal tubules	–	Fox and Duronio, 2013
	Epidermis	–	Fox and Duronio, 2013	
	Mechanosory bristle/shaft cells	–	Audibert et al., 2005	
	Mechanosory bristle/socket cells	–	Audibert et al., 2005	
	<i>Calliphora erythrocephala</i>	Ovary/nurse cells	–	Ribbert, 1979
	<i>Aedes aegypti</i>	Midgut	Immune response	Ray et al., 2009; Maldonado et al., 2019
	<i>Culex pipiens</i>	Midgut	Immune response	Ray et al., 2009; Maldonado et al., 2019
<i>Anopheles gambiae</i>	Ovary/nurse cells	–	Zhimulev, 1996	
<i>Anopheles albimanus</i>	Ovary/nurse cells	–	Zhimulev, 1996	
<i>Chironomus tentans</i>	Salivary glands	–	Zhimulev et al., 2004	
Orthoptera	<i>Locusta migratoria</i>	Fat body (female)	Massive synthesis of vitellogenin	Guo et al., 2014; Wu et al., 2016; Wu et al., 2018
		Fat body (male)	–	Ren et al., 2019
		Fat body-like tissue	–	Ren et al., 2019
		Ovary/follicle cells	Oocyte maturation and egg chamber development	Wu et al., 2016; Wu et al., 2018
Coleoptera	<i>Tribolium castaneum</i>	Midgut/intestinal stem cells	–	Parthasarathy and Palli, 2008
Lepidoptera	<i>Ephestia kuehniella</i>	Malpighian tubules	–	Buntrock et al., 2012
		Silk glands	High silk production	Buntrock et al., 2012
		Wing epithelium	Increase cell size	Edgar and Orr-Weaver, 2001
	<i>Spodoptera frugiperda</i>	Ovary	–	Meneses-Acosta et al., 2001
Hymenoptera	<i>Apis mellifera</i>	Malpighian tubules	–	Rangel et al., 2015
		Brain	–	Rangel et al., 2015
		Stinger	–	Rangel et al., 2015
		Leg	–	Rangel et al., 2015
		Thoracic muscle	–	Rangel et al., 2015
		Flight muscle	–	Rangel et al., 2015
	<i>Bombus terrestris</i> L.	Mandibular muscle (haploid male)	Keep pace with females in terms of muscular metabolic activity and efficiency	Aron et al., 2005
		Thoracic muscle (haploid male)	Keep pace with females in terms of muscular metabolic activity and efficiency	Aron et al., 2005
		Leg muscles (haploid male)	Keep pace with females in terms of muscular metabolic activity and efficiency	Aron et al., 2005
		Whole body	Body size and behavior	Scholes et al., 2013
<i>Pogonomyrmex badius</i>	Whole body	Body size and behavior	Scholes et al., 2013	
<i>Camponotus floridanus</i>	Whole body	Body size and behavior	Scholes et al., 2013	
<i>Atta texana</i>	Whole body	Body size and behavior	Scholes et al., 2013	

growing neurons, while simultaneously maintaining the blood-brain barrier, which otherwise would be disrupted through cell division (Unhavaithaya and Orr-Weaver, 2012; Zülbahar et al., 2018). During *Drosophila* pupal development, shaft and socket cells that form parts of the mechanosory bristle undergo two or three endocycles to produce cells with 8C or 16C DNA (Audibert et al., 2005). Therefore, the extensive cell polyploidy in *Drosophila* variant tissues and developmental stages displays the cellular potentials to remodel for various functions.

Polyploidy cells are also found in variant complete metamorphosis insect species. In mosquitoes, polyploidy cells arise in the anterior and posterior midgut of *Aedes aegypti*, yet only in anterior midgut of *Culex pipiens* during larval development (Ray et al., 2009). These polyploid midgut cells facilitate the fast production of immune proteins in a process known as priming (Maldonado et al., 2019). Polyploid chromosomes are formed in ovarian nurse cells of *Anopheles albimanus* and polytene chromosomes are formed in ovarian nurse cells of *Anopheles gambiae* and salivary glands of *Chironomus tentans* (Zhimulev, 1996; Zhimulev et al., 2004). In the *Calliphora erythrocephala*, nurse cells also developed polytene chromosomes post inbreeding and artificial selection (Ribbert, 1979). In the honey bee *Apis mellifera*, the Malpighian tubules is the most highly polyploid secretory cells, and the brain, stinger, leg, thoracic muscle, and flight muscle also generate polyploid cells (Rangel et al., 2015). In the bumble bee *Bombus terrestris* L., cells in mandibular, thoracic, and leg muscles of the haploid male undergo one round of endoreduplication to become functionally diploid and get comparable size and function to those of the diploid female (Aron et al., 2005). Ploidy levels between and among worker castes of four highly polymorphic ant species, red imported fire ant *Solenopsis invicta*, Florida harvester ant *Pogonomyrmex badius*, Florida carpenter ant *Camponotus floridanus*, and Texas leaf-cutting ant *Atta texana*, are positively related to worker size, suggesting that worker task performance might benefit from endoreduplication of certain tissues (Scholes et al., 2013). In the red flour beetle *Tribolium castaneum* larval stages, intestinal stem cells (ISC) conduct endoreduplication for adult midgut polyploid epithelium formation (Parthasarathy and Palli, 2008). In the flour moth *Ephesia kuehniella*, cells of Malpighian tubules and silk glands undergo endocycle through all larvae instars, and even, in the last instar, larvae nuclei are polyploid with a high DNA content, provoking a branched nucleus (Buntrock et al., 2012). In *Spodoptera frugiperda* sf9 ovarian cells, cell cycle arrests in G2/M phase to generate polynucleated cells (Meneses-Acosta et al., 2001).

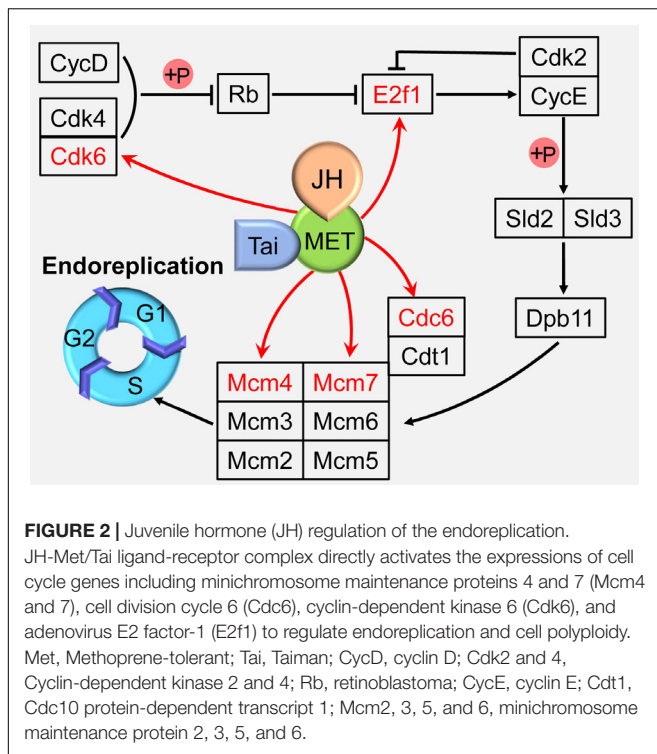
In the migratory locust, *Locusta migratoria*, an incomplete metamorphosis insect species, fat body cells of female adults undergo extensive DNA replication to produce from 4C to 64C polyploidy cells during vitellogenesis. These polyploidy cells support the rapidly massive synthesis of vitellogenin, the main proteins in egg maturation, for dozens of oocytes in every gonadotrophic cycle. Besides, female locust follicle cells also undergo high levels of endoreduplication during oocyte maturation to synthesize chorion protein for success chorionogenesis

(Kimber, 1980; Guo et al., 2014; Wu et al., 2016, 2018). While in the male locust, the abdominal fat body (FB) undergoes endocycle and generates 4C to 8C polyploidy cells, while the fat body-like tissue (FLT) surrounding testis follicles is dominated by diploid cells instead of polyploidy cells (Ren et al., 2019). Thus, similar or same tissues in one species display different polyploidy levels between both genders or in different hemocoel locations.

Both polyploid and polytene chromosomes originate by the endocycle. In the polytene chromosomes, the replicated sister chromatids remain attached and aligned and the chromosomes become visible. In polyploid cells, the replicated chromosome copies are dispersed and the chromosomes are not visible in the nucleus (Frawley and Orr-Weaver, 2015). Therefore, polytene chromosomes are specialized polyploid chromosomes. Transition between polytene and polyploid chromosome have been studied in *Drosophila* ovarian nurse cells (Bauer et al., 2012). Condensin II drives axial compaction and therefore force apart chromatids destroying a typical polytene chromosome structure, thus, polyploid chromosome are formed in the nurse cells (Bauer et al., 2012). It seems that polytene chromosomes formation are energy-saving to mainly skip duplicating heterochromatic regions, while polyploid chromosomes are advantageous to massively duplicate any gene upon necessity although its formation needs more energy (Frawley and Orr-Weaver, 2015; Stormo and Fox, 2017). Thus, we speculated that the use of different endoreduplication strategies might indicate an adaptive trade-off between energy consuming in DNA amplification and the tissue demands for specific protein syntheses. Taken together, the extensive occurrence of cell polyploidy in variant insect species provide excellent study materials to decipher the regulatory mechanisms of endocycle in the light of evolution.

GENETIC NETWORK IN ENDOCYCLE

Although significantly different from the conventional cell cycle, endocycle uses regulatory pathways that also function in diploid cells (Lee et al., 2009; Calvi, 2013). Cyclin-dependent kinase (Cdk) 4/6 form an active complex with Cyclin D (CycD), phosphorylate and inactivate retinoblastoma protein (Rb), releasing transcription factor adenovirus E2 factor-1 (E2f1) from Rb-mediated repression (Swanson et al., 2015; Tigan et al., 2016). Cyclin E (CycE), which is activated by E2f1, together with cyclin-dependent kinase 2 (Cdk2), triggers S-phase by phosphorylating Sld2 and Sld3, which then recruit Dpb11 to replication origins, thereby activating the Mini-chromosome maintenance (Mcm) 2–7 DNA helicase (Zielke et al., 2011; Tanaka and Araki, 2012; Bell and Kaguni, 2013). Together with the origin recognition complex and Cdt1 (Cdc10 protein-dependent transcript 1), Cdc6 loads Mcm proteins onto DNA at the origins of replication to facilitate the formation of stable prereplication complex in G1 phase, thereby licensing these sites to initiate DNA replication in S phase (Sun et al., 2013; Wu et al., 2016; **Figure 2**).



ENDOCYCLE GOVERNED BY INSECT HORMONE

Juvenile hormone (JH) and 20-hydroxyecdysone (20E), as two dominant hormones that are involved in insect development, metamorphosis, and reproduction, play key roles in the regulation of cell polyploidy by binding to their respective nuclear receptors to initiate the expression of cell cycle genes (Fallon and Gerenday, 2010; Jindra et al., 2013; Yamanaka et al., 2013; Guo et al., 2014; Moriyama et al., 2016; Wu et al., 2016, 2018; Maldonado et al., 2019). Many studies have confirmed that the two hormones can regulate cell polyploidy in insects.

Juvenile hormone is a sesquiterpenoid produced by the corpora allata, and functions in cells by inducing the heterodimerization of its receptor, Methoprene-tolerant (Met) with Taiman (Tai). Met and Tai form a functional receptor to control insect development, metamorphosis, and reproduction (Jindra et al., 2013; Wang et al., 2017). The migratory locust, *Locusta migratoria* is the most well-studied insect species in the JH regulation on cell polyploidy. The adult fat body undergoes extensive DNA replication to highly polyploidy cells during vitellogenesis for successful reproduction under the regulation of JH (Nair et al., 1981; Oishi et al., 1985). In an effort to elucidate the mechanisms of JH action on female reproduction, digital gene expression profiling was employed to identify differentially expressed genes in JH-deprived fat bodies and those further treated with a JH analog (JHA). DNA replication pathway was identified as the top one “hit” after JHA treatment by Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses (Guo et al., 2014). Further study revealed that Met binds directly on

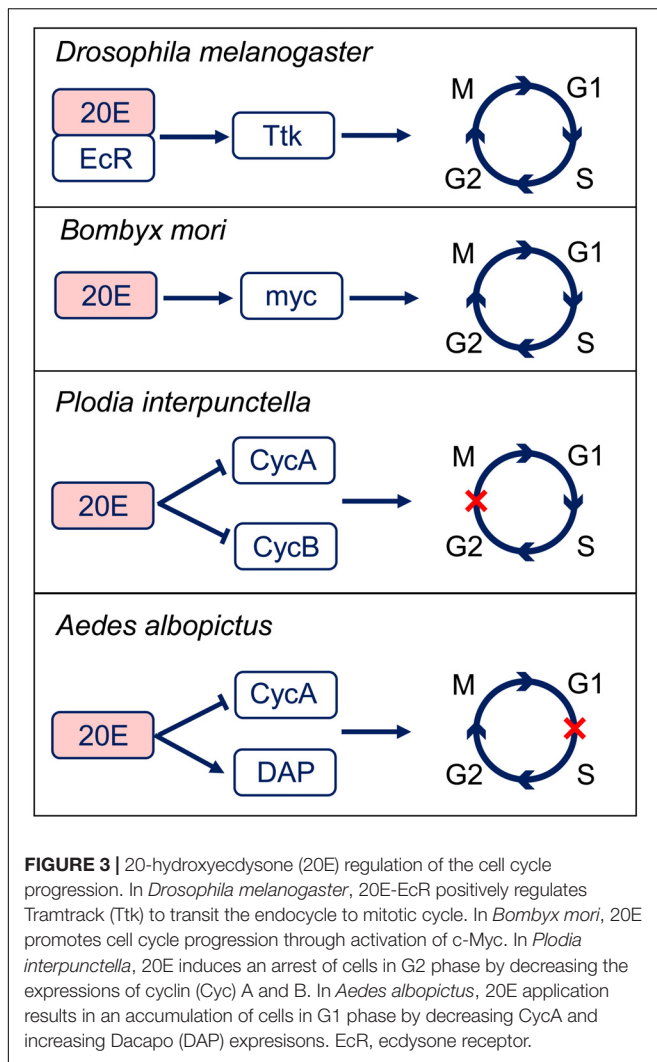
the E-box (CACGTG) or E-box-like (CACGCG) motifs in the promoter regions of *Mcm4*, *Mcm7*, and *Cdc6* to activate the transcription of these genes, which promotes the endoreplication of fat body cells (Guo et al., 2014; Wu et al., 2016). In addition, JH-Met/Tai complex directly activates the transcription of *Cdk6* and *E2f1*, and depletion of *Cdk6* or *E2f1* results in significantly decreased the cell polyploidy level, precocious mitotic entry for multinuclear appearance, and increased cell numbers in the fat body cells (Wu et al., 2018; Figure 2). These results indicate that JH is able to directly activate several cell cycle genes to enhance endoreplication process.

The ecdysteroid is also involved in cell cycle regulation in a different way from JH. The major ecdysteroid in insects such as 20E not only promotes molting at juvenile stages but also affects lifespan, learning, stress-induced responses, sleep regulation, social interactions, and sexual behavior in adults (Yamanaka et al., 2013). 20E mediate the switch between endocycle and site-specific endoreplication by binding to the ecdysone receptor (EcR) (Maldonado et al., 2019). Importantly, 20E also regulates DNA replication and polyploidy during insect metamorphosis (Sun et al., 2008). Correlations between epidermal DNA synthesis and hemolymph ecdysteroid levels have been revealed in the tobacco hornworm *Manduca sexta* and *Calpodes ethlius* (Wielgus et al., 1979; Dean et al., 1980). In *Plodia interpunctella*, imaginal wing cells arrest in G2 phase post 20E treatment by inducing a sharp decrease in the levels of cyclin A and B expression (Mottier et al., 2004). In cell line C7-10 from the mosquito *Aedes albopictus*, 20E application resulted in cell arrest in the G1 phase (Gerenday and Fallon, 2004). Further studies suggest that 20E treatment increases the expression of Cyclin E-Cdk2 inhibitory protein DACAPO, while decreases the expression of cyclin A (Fallon and Gerenday, 2010). In the wing discs of silkworm *Bombyx mori*, 20E directly activates c-Myc transcription, which subsequently stimulates the expression of cell cycle core regulators, including cyclinA, cyclinB, cyclinD, cyclinE, Cdc25, and E2F1 genes to promote endocycle progression and cell polyploidization during metamorphosis (Moriyama et al., 2016; Figure 3). These studies indicate that 20E promotes cell proliferation in low titer and initiates cell cycle arrest in high titer (Koyama et al., 2004; Moriyama et al., 2016). Thus, actions of 20E on cell polyploidy vary in different insect species in a dose-dependent way.

However, the interactions between JH and 20E in cell polyploidy regulation are still largely unknown. Classically, 20E counteracts on the function of JH during molting and metamorphosis (Liu P. C. et al., 2018; Liu S. et al., 2018). Studies of JH and 20E actions on cell polyploidy focus mainly on the aspects of DNA reduplication enhancement and cell division arrest, respectively. JH and 20E may jointly coordinate the timing of DNA reduplication and cell division during MES process.

ENVIRONMENT-EVOKED ENDOCYCLE

In response to pathogen, wound, and aging, some tissue cells are re-programmed, exiting their mitotic cell cycle to differentiate into polyploidy cells (Zielke et al., 2013). In *Drosophila*, ovarian pseudonurse cells raised the frequency of polytene chromosomes



in response to low temperature and protein-rich food (Mal'ceva et al., 1995). In *Anopheles albimanus*, the increase of cell DNA synthesis after challenged with different microorganism and *Plasmodium sp.* is considered as an adaptive immune response, which can be recalled quickly in next exposure to the same pathogens (Hernández-Martínez et al., 2013; Contreras-Garduño et al., 2015). In the adult *Drosophila* epidermis and hindgut, post-mitotic differentiated diploid cells respond to wounding by entering the endocycle, and this process is called wound-induced polyploidization (WIP) (Losick et al., 2016). In the honey bee *Apis mellifera*, endoreplication levels change with worker age in a tissue-specific manner, and there is a surprisingly significant decrease in cell ploidy in the leg and the thoracic muscles with aging (Rangel et al., 2015).

On the other hand, the mitotic cell cycle can be activated in some insects post stimuli. In *Aedes albopictus*, midgut generates an increase in regenerative cells after chemical and bacterial damages (Janež et al., 2017). Besides, cell proliferation has been tested in *Aedes aegypti* midgut post viral infections and oxidative stress (Taracena et al., 2018). In *Drosophila*, starvation

reduces E2f1 mRNA levels and blocks endocycling in salivary glands, whereas overexpression of E2f1 restores endocycles in starved fruit flies (Zielke et al., 2011). Inhibition of the target of rapamycin (TOR) in *Drosophila* prothoracic gland causes nutrient-dependent endocycle inhibition and developmental arrest (Ohhara et al., 2017). Thus, cell polyploidy displays adaptive plasticity to respond to unpredicted environment changes through remodeling the mitotic cycle to endocycle.

MOLECULAR MECHANISMS OF MITOSIS/ENDOCYCLE SWITCH

Cells switch from the mitotic cycle to the endocycle response to developmental signals or environmental stimuli, and become polyploidy rather than proliferation. This intermediate process is defined as the mitosis/endocycle switch (MES). The thrombopoietin and the Notch pathways have been identified as the major regulators of the mitotic-to-endocycle switch. The thrombopoietin pathway acts during differentiation of megakaryocytes, and the Notch pathway acts during the oogenesis and differentiation of trophoblasts (Zimmet and Ravid, 2000; Deng et al., 2001; López-Schier and St Johnston, 2001). Besides, the JNK pathway is also demonstrated to be required to promote mitosis prior to the transition, independent of the cell cycle components acted on by the Notch pathway (Jordan et al., 2006). In follicle cells, the Notch pathway stops proliferation and promotes a switch from the mitotic cycle to the endocycle (Sun and Deng, 2007). In contrast to Notch signal, Hedgehog (Hh) signal appears to promote follicle cell proliferation (Zhang and Kalderon, 2000).

The Notch signaling pathway in *Drosophila* follicle epithelium is a key upstream regulator of the MES. The ligand Delta expressed by oocytes activates the Notch receptor in follicle cells (Deng et al., 2001). Notch activity leads to downregulation of String (STG) and Dacapo (DAP), and upregulation of Cdh1/Fizzy-related (FZR) (Jordan et al., 2006). STG is a G2/M regulator Cdc25 phosphatase that removes inhibitory phosphates from Cdk1, allowing it to associate with CycA and CycB and initiate mitosis (Zielke et al., 2013). CycB-Cdk1 in return activates the STG by phosphorylation, which creates a positive feedback loop (Zielke et al., 2013). The activity of CycB-Cdk1 is further inactivated through phosphorylation (Welburn et al., 2007). Cdk1 can be phosphorylated on Tyr15 by Wee1 kinase, and on both Thr14 and Tyr15 by Myelin transcription factor 1 (Myt1) kinase (Parker and Piwnicka-Worms, 1992; Mueller et al., 1995). Interestingly, Wee1 is phosphorylated and thereby inactivated by CycB-Cdk1, which creates a double negative feedback loop (Tang et al., 1993). CycA/CDK directly activates the Myb-MuvB (MMB) complex to induce transcription of a battery of cell cycle genes required for mitosis. AuroraB (AurB) is an MMB regulated gene, and knockdown of AurB and other subunits of the chromosomal passenger complex (CPC) induced endoreplication (Rotelli et al., 2019). Down-regulating of the CycE/CDK complex inhibitor DAP releases CycE/Cdk2, thus blocks S phase initiation (Zielke et al., 2013). FZR is a regulator of the APC ubiquitination complex that degrade CycA and CycB,

thereby reinforcing the block to mitosis (Zielke et al., 2013). FZR is negatively regulated by the homeodomain gene Cut, but Cut does not seem to regulate String (Stg) (Sun and Deng, 2005, 2007). String/Cdc25 and the transcription factor Cut are repressed by a zinc-finger transcription factor Hindsight (Hnt) (Sun and Deng, 2007). However, during oogenesis, Notch in *Drosophila* follicle cells is down-regulated and cooperate with Tramtrack (Ttk), a transcription factor that induces endocycle exit and entry in site-specific endoreplication (Sun et al., 2008). In the *Drosophila* bristle lineage, Ttk downregulates Cyclin E expression and is probably involved in the exit of the cells from the cell cycle (Audibert et al., 2005; **Figure 4**).

Hippo pathway in the *Drosophila* nervous system has been reported to be involved in the MES. Yki, an essential transcriptional activator of Hippo pathway, is required to establish the correct ploidy and is partly post-transcriptionally regulated miR-285. miR-285 is an upstream regulator of the Hippo pathway, which can directly target Yki cofactor Multiple Ankyrin repeats Single KH domain (Mask) to suppress Yki activity and down-regulates the expression of its downstream target cyclin E (CycE). Disturbance of CycE expression in subperineurial glial cell (SPG) causes abnormal endoreplication, which leads to aberrant DNA ploidy and defective septate junctions (Li et al., 2017). Moreover, the N-terminal asparagine amidohydrolase homolog Öbek counteracts the activity of Yki, as well as the activity of the fibroblast growth factor (FGF) receptor Heartless in glial cells to limit endoreplication and the consequent appearance of extra nuclei. However, other dividing cells are not affected by alteration of Öbek expression (Zülbahar et al., 2018). Cooperative activation of Yki and JNK

upregulates *Drosophila* inhibitor-of-apoptosis protein 1 (Diap1) prevents mitotic entry by downregulating G2/M cyclin CycB, thereby inducing endoreplication (Cong et al., 2018). The protein level of *Drosophila* CycB is mediated by ubiquitination-mediated degradation, and Diap1 contains a RING domain and acts as an E3 ligase that could cause ubiquitination-mediated degradation of CycB in either direct or indirect manner (Xu et al., 2009; Shabbeer et al., 2013; Cong et al., 2018; **Figure 4**).

A recent study in the migratory locust *Locusta migratoria* reported that FB cells undergo endocycle and become polyploidy, while FLT cells undergo rounds of mitotic cycle and dominated by diploid cells at the same developmental stages during male adult maturation. FB-FLT cells displayed different cell cycle strategy at the same developmental stages, avoiding the interference of developmental programming. Further analyses of comparative transcriptomes of FB-FLT provided valuable candidate cell cycle genes and transcription factors to investigate the molecular mechanism of MES (Ren et al., 2019). Probably, the FB-FLT of locust is one of novel promising systems to study MES.

The developmental signals and environmental stimuli can affect the performance of the MES. In endocycling cells, promoters of pro-apoptotic genes are silenced rendering them insensitive to DNA damage (Mehrotra et al., 2008). Starvation induces the pause of the M/E switch in *Drosophila* follicle cells, blocking the entry of egg chambers into vitellogenesis. Paused MES is induced by a reduction of insulin signal and involves a previously unknown crosstalk among FoxO, Cut, and Notch. FoxO is dispensable for the normal M/E switch. However, in paused MES, FoxO activates Cut expression cell-autonomously in follicle cells (Jouandin et al., 2014). Besides, MES regulation may vary in different tissues or cell types. The mitotic cyclins in endocycling follicle cells are regulated only by ubiquitin-dependent degradation, whereas in salivary glands, transcription of mitotic cyclins is terminated concomitantly with up-regulation of Fzr (Zielke et al., 2008; Maqbool et al., 2010). The suppression of mitotic regulators in endoreplicating salivary glands is mediated, at least in part, by the transcriptional repressor E2F2 (Zielke et al., 2011). Besides, Notch controls the switches of different cell cycle programs in follicle cells, yet the nurse cell endocycles are normal in Notch mutant clones (Lilly and Duronio, 2005).

In brief, *Drosophila* follicle cells and glial cells are two well-studied systems and locust FB-FLT would be a novel promising system to investigate the molecular mechanisms of MES (Sun and Deng, 2007; Li et al., 2017; Cong et al., 2018; Zülbahar et al., 2018; Ren et al., 2019). Developmentally controlled MES were regulated by Notch and Hippo signaling pathway; however, the environmental-stimulated MES have probable specific mechanisms which are condition-dependent.

PROSPECTS

Insects are the best models to study regulatory mechanisms of polyploidy, compared to vertebrates and plants. First, polyploidization can be observed at larvae and adult stages in both complete and incomplete metamorphosis insects.

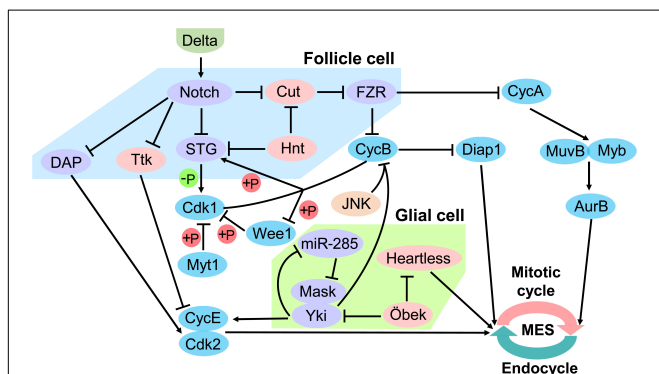


FIGURE 4 | Regulation of mitotic/endocycle switch. In the *Drosophila* follicle cell, Notch signaling pathway is a key upstream regulator of the mitotic/endocycle switch (MES). Activated Notch protein by the ligand Delta from oocytes terminates follicle cell proliferation and promotes MES by downregulating String (STG) and Dacapo (DAP) and upregulating FZR. In the *Drosophila* glial cell, Hippo signaling pathway is involved in MES. miR-285 suppresses Yki-Mask dimer activity by targeting Mask to downregulate cyclin E (CycE). Moreover, the N-terminal asparagine amidohydrolase homolog Öbek counteracts the activity of Yki and Heartless to limit endoreplication. FZR, Cdh1/Fizzy-related; Yki, Yorkie; Mask, Multiple Ankyrin repeats Single KH domain; Hnt, Hindsight; Ttk, Tramtrack; Cdk1 and 2, Cyclin-dependent kinase 1 and 2; Myt1, Myelin transcription factor 1; CycA and B, cyclin A and B; Diap1, *Drosophila* inhibitor-of-apoptosis protein 1; AurB, AuroraB.

Second, cells in different tissues of insects undergo endocycle during particular developmental stages or response to environmental stimuli.

Insect hormones play critical roles on cell polyploidy progression. JH promotes cell polyploidization by directly upregulating several DNA replication genes (Guo et al., 2014; Wu et al., 2016, 2018). However, whether JH also acts on cell division genes remains to be studied. Besides, the role of 20E played in cell cycle regulation is unclear, and the target genes of 20E in cell cycle process are also largely unknown. In some processes, JH plays a critical role in defining the action of 20E, and 20E also affects the function of JH (Jia et al., 2017; Liu P. C. et al., 2018; Liu S. et al., 2018). In addition, JH and 20E are reported to regulate MES-related genes and miRNA (Gerenday and Fallon, 2011; Dong et al., 2015; Wang et al., 2016; Song et al., 2019). Therefore, the crosstalk of JH and 20E in cell polyploidy regulation through MES need to be further investigated to clarify their biological functions and adaptive mechanisms to developmental programming and environmental changes.

Because MES is regulated by several canonical pathways, further studies should be conducted to decipher the relationships

of these pathways and their interactions orchestrated by hormones. In addition, we attempt to answer whether these pathways are conserved in the regulation on MES in different tissue cells and diverse insect species. Therefore, studies of cell cycle genes and related pathways will shed light on the evolution of cell polyploidy and provide more target genes for pest control.

AUTHOR CONTRIBUTIONS

LK, WG, and DR designed the research. DR, JS, and MN collected the references. DR, JS, WG, and LK wrote the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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