Endoreplication

Norman Zielke¹, Bruce A. Edgar¹, and Melvin L. DePamphilis²

¹Deutsches Krebsforschungszentrum (DKFZ)-Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH) Allianz, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany

²National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892-2753

Correspondence: b.edgar@Dkfz-Heidelberg.de; n.zielke@DKFZ-heidelberg.de

Developmentally programmed polyploidy occurs by at least four different mechanisms, two of which (endoreduplication and endomitosis) involve switching from mitotic cell cycles to endocycles by the selective loss of mitotic cyclin-dependent kinase (CDK) activity and bypassing many of the processes of mitosis. Here we review the mechanisms of endoreplication, focusing on recent results from *Drosophila* and mice.

Eukaryotic cells proliferate by undergoing a sequence of events termed the “mitotic cell cycle” in which the genome is duplicated once and only once between cell divisions. The result is a population of cells with two copies of each chromosome (diploid, or 2C). Agents that interfere with the mechanisms that govern genome duplication frequently induce reinitiation of nuclear DNA replication during S phase. This phenomenon, termed “DNA rereplication,” is an aberrant event that produces a population of cells with a heterogeneous DNA content that reflects incomplete chromosome duplication, stalled replication forks, and DNA damage. In most cells, these events can lead to inducing the cell’s DNA damage response and can lead to apoptosis (Lee et al. 2010).

Remarkably, some cells are developmentally programmed to exit their mitotic cell cycle in response either to environmental signals or to injury or stress, and then differentiate into nonproliferating, viable, polyploid cells. This

phenomenon, termed “developmentally programmed polyploidy,” is a normal part of animal and plant development that occurs frequently in ferns, flowering plants, mollusks, arthropods, amphibians, and fish, although rarely in mammals. In contrast to DNA rereplication, developmentally programmed polyploidy produces cells with a DNA content of $>4C$, but in integral multiples of $4C$ (e.g., $8C$, $16C$, $32C$, etc.), consistent with multiple S phases in the absence of cytokinesis. These cells typically stop proliferating but remain viable in a terminally differentiated state that may serve to regulate tissue size or organization, to trigger cell differentiation or morphogenesis, to increase the number of genes dedicated to tissue-specific functions without increasing the number of cells, or to adapt to environmental conditions. Mitotic divisions of polyploid cells are common for plant species, but they are rarely found in animals. Although known for decades, polyploid mitosis in insects remained mostly unstudied until it was recently

N. Zielke et al.

shown that the cells of the rectal papilla in *Drosophila* undergo mitosis after executing two or more endocycles (Fox et al. 2010). Thus, polyploidy is not an irreversible process, although the benefit of this cell cycle variant remains to be elucidated.

Developmentally programmed polyploidy occurs by at least four different mechanisms (Ullah et al. 2009). Proliferating cells in the syncytial blastoderm of *Drosophila* embryos and some hepatocytes in the postnatal liver of mammals become multinucleated and therefore polyploid by failing to undergo cytokinesis after mitosis (“acytokinetic mitosis”). Differentiation of skeletal muscle myoblasts into myotubes, monocytes into osteoclasts, and formation of placental syncytiotrophoblasts involves “cell fusion” to produce multinucleated, terminally differentiated cells that are similarly polyploid. Alternatively, cells may exit their mitotic cell cycle by arresting mitosis during anaphase and failing to undergo cytokinesis. This phenomenon, termed “endomitosis,” produces cells with a single giant nucleus that may subsequently fragment into a multinuclear appearance. Endomitosis occurs in mammals when megakaryoblasts differentiate into megakaryocytes (Bluteau et al. 2009), and in some plant cells (Weingartner et al. 2004). However, the primary mechanism for developmentally programmed polyploidy in arthropods (Smith and Orr-Weaver 1991; Edgar and Orr-Weaver 2001), plants (de la Paz Sanchez et al. 2012), and possibly mammals (Ullah et al. 2009) is “endoreplication” (also referred to as “endoreduplication”). Endoreplication occurs when a cell exits the mitotic cell cycle in G₂ phase and undergoes multiple S phases without entering mitosis and undergoing cytokinesis. The result is a giant cell with a single, enlarged, polyploid nucleus.

ENDOCYCLING CELL TYPES

“Endocycles” refer to multiple rounds of nuclear genome duplication in cells undergoing either endoreplication or endomitosis. Endocycles typically include Gap (G) phases between each S phase and use the same molecular machinery as mitotic cell cycles to regulate successive

rounds of DNA replication. In *Drosophila*, larval growth is achieved primarily via endoreplication (Edgar and Nijhout 2004). Consequently, most larval tissues are composed mainly of polyploid cells. These tissues include the salivary glands, fat body, epidermis, gut, trachea, and Malpighian or renal tubules (Lee et al. 2009a). The best studied of these are the giant salivary gland cells that undergo about 10 endocycles, resulting in a final ploidy of ~1350C (Hammond and Laird 1985b). In the female germline, nurse cells, which provide the developing oocyte with mRNA and proteins, achieve a final ploidy of ~1500C (Hammond and Laird 1985a), whereas the somatic follicle cells that surround the egg undergo only three endocycles. Loss of the ability to endoreplicate in nurse cells results in sterility, indicating that endocycling is essential for oogenesis (Lilly and Spradling 1996). During pupal development, shaft and socket cells that form parts of the mechanosensory bristle undergo two or three endocycles to produce cells with 8C or 16C DNA (Audibert et al. 2005). Finally, the subperineurial glia increases in size by endoreplication, allowing it to accommodate growing neurons, while simultaneously maintaining the blood–brain barrier (Unhavaithaya and Orr-Weaver 2012).

Mouse development includes many examples of terminal cell differentiation (Ullah et al. 2009), but in only four of these cases does polyploidy occur through endoreplication. Cardiac myoblasts, basal epithelial cells, and primitive podocytes endoreplicate when placed under stress by either injury or infection. Trophoblast stem (TS) cells are the paradigm for endoreplication in mammals (Hu and Cross 2010). They are derived from the trophectoderm of blastocysts, the outer layer of epithelial cells that give rise exclusively to the various cells composing the placenta. TS cells proliferate in vitro when cultured in the presence of fibroblast growth factor 4 (FGF4) and conditioned medium, but when cultured without these factors, they differentiate into the trophoblast giant (TG) cells that are essential for implantation and placental development. When reestablished in a blastocyst, TS cells contribute exclusively to the placenta, giving rise to all of the cell lineages derived from

the trophoderm (Oda et al. 2010). However, the DNA content of TG cells generated in vitro seldom exceeds 64C, whereas TG cells in vivo have been reported to reach >1000C.

SUSTAINING ENDOCYCLES

Oscillation of Cyclin E-Cdk2 Activity Drives Endocycles

Cyclin E (CycE) and its kinase partner cyclin-dependent kinase 2 (Cdk2, encoded by *cdc2c*) are the major regulators of S-phase entry in *Drosophila*. In *cycE*^{-/-} embryos, DNA synthesis is abolished in cells undergoing mitotic cell cycles or endocycles (Knoblich et al. 1994). Similarly, endocycles in salivary glands are eliminated by tissue-specific removal of Cdk2/*cdc2c* (Zielke et al. 2011). Moreover, overexpression of CycE is sufficient to induce DNA replication in endoreplicative cells arrested by starvation (Britton and Edgar 1998). Likewise, ablation of both cyclin E (CcnE) alleles in mice prevents endocycles in TG cells and megakaryocytes (Geng et al. 2003; Parisi et al. 2003; Eliades et al. 2010), and ablation of Cdk2 prevents endoreplication in TS cells (Ullah et al. 2008). Together, these results showed that CycE/CcnE-Cdk2 is essential in flies and mammals for the execution of S phases during endocycling. Studies in yeast and frog eggs (Bell and Kaguni 2012; Tanaka and Araki 2012) revealed that CycE-Cdk2 triggers S phase by phosphorylating Sld2 and Sld3, which then recruit Dpb11 to replication origins, thereby activating the Mcm2-7 DNA helicase. Mammalian orthologs of Sld2, Sld3, and Dpb11, and *Drosophila* orthologs of Sld2 and Dpb11 have been identified (see Supplemental Table 3 online), suggesting that the same mechanism applies to these organisms as well.

Continuous overexpression of CycE blocks endocycles in *Drosophila* salivary glands (Follette et al. 1998; Weiss et al. 1998), implying that the oscillation of CycE-Cdk2 activity is essential for endocycle progression. CycE is an unstable protein (Lilly and Spradling 1996; Weng et al. 2003) that is targeted for ubiquitin-dependent degradation by the E3 ubiquitin ligase CRL1-Ago (Moberg et al. 2001). The product

of the *Drosophila minus (mi)* gene—not conserved in vertebrates—physically interacts with CRL1-Ago and confers its substrate specificity toward CycE (Szuplewski et al. 2009). Salivary gland and follicle cells that are either *ago*^{-/-} or *mi*^{-/-} fail to undergo DNA replication, implying that the degradation of CycE is essential for endocycle progression (Shcherbata et al. 2004; Szuplewski et al. 2009; Zielke et al. 2011).

In mammals, cyclin E is phosphorylated by cyclin A (CcnA)-Cdk2 soon after S phase begins during a mitotic cell cycle. This phosphorylated form of CcnE is then ubiquitinated by CRL1-Fbw7 and degraded by the 26S proteasome (Koepp et al. 2001; Strohmaier et al. 2001). Moreover, *Fbw7*^{-/-} mouse embryos and placentas have increased levels of cyclin E protein, which persist throughout G and S phases (Tetzlaff et al. 2004), suggesting that this function is conserved between flies and mammals. However, there is no evidence that CRL1-Fbw7 activity oscillates during either mitotic cell cycles or endocycles. Therefore, its function may be to render CycE constitutively unstable, rather than to function as part of the endocycle core oscillator.

A “Core Oscillator” for Endocycles

Why CycE-Cdk2 activity must oscillate in the endocycle and how this oscillation is orchestrated have been critical questions driving recent research on *Drosophila* endoreplication. In metazoa, S phase depends on many genes that rely on the E2F transcription factors for their expression (van den Heuvel and Dyson 2008). Cyclin E is one such gene. Mammals contain nine E2F proteins, three of which activate transcription by forming heterodimeric complexes with DP proteins that allow site-specific binding to DNA. The smaller fly genome contains only a single activator termed E2F1 and a single repressor, E2F2, both of which dimerize with the same DP protein. Activator E2FDP complexes are required for cyclin E expression in both mammals and flies.

E2F1 is essential for both the expression and oscillation of CycE protein in *Drosophila* (Zielke et al. 2011, and references therein).

N. Zielke et al.

Consequently, the deletion of E2F1 causes rapid endocycle arrest in *Drosophila* cells. Whereas E2F1 transcripts are expressed ubiquitously in actively cycling cells, E2F1 protein accumulates during G phases and is destroyed during S phase. This oscillation of E2F1 protein is mediated by the CRL4-Cdt2 E3 ubiquitin ligase, whose activity requires ongoing DNA replication (Arias and Walter 2006; Shibutani et al. 2007, 2008). S-phase-specific degradation of E2F1 relies on the fact that activation of CRL4-Cdt2 requires chromatin-bound PCNA, which exists only at active replication forks (Havens and Walter 2009). Substrates of CRL4-Cdt2 contain a conserved PCNA-interacting protein (PIP) motif that mediates the interaction between PCNA and its substrate (Arias and Walter 2006). Consequently, mutation of the amino-terminal PIP box in E2F1 inhibits its turnover (Shibutani et al. 2008). Tellingly, either depletion of CRL4-Cdt2 or stabilization of E2F1 by PIP box mutations prevents endocycling in salivary glands (Zielke et al. 2011). Therefore, E2F1 and CRL4-Cdt2 are essential components of an autoregulatory feedback loop that mediates the oscillations of CycE activity, which drive endocycling in *Drosophila* salivary glands (Fig. 1).

Although both E2F and CRL4-Cdt2 are also essential for endoreplication in *Arabidopsis* (Roodbarkelari et al. 2010), the periodic destruction of E2F1 by CRL4-Cdt2 has not yet been shown in either mammals or plants, and therefore the E2F1–CRL4-Cdt2 oscillator proposed by Zielke and coworkers may be unique to flies or perhaps arthropods in general. As previously noted, an E2F-independent S-phase oscillator is theoretically possible. This might comprise CRL4-Cdt2 and another of its well-characterized substrates, such as the Cdt1 ortholog *double-parked* (Dup), an essential component of the origin licensing mechanism (Zielke et al. 2011).

Given that endoreplicating cells generally grow to giant proportions (Britton and Edgar 1998; Pierce et al. 2004), they are presumably dependent on extracellular growth signals. Consistent with this hypothesis, E2F1 protein accumulates in salivary glands in which the nutri-

Figure 1. Sustaining endocycles in *Drosophila* salivary glands. (A) Schematic of a larval salivary gland with attached fat body. Both salivary glands and fat bodies undergo endocycles resulting in cells with DNA contents of $\sim 1350C$ and $\sim 256C$, respectively, whereas the diploid cells of the imaginal ring proliferate mitotically. (B) Diagram of the regulatory network proposed to control salivary gland endocycles. The counterplay of E2F1 and its antagonist CRL4-Cdt2 ensures that CycE activity peaks in late G phase. This initiates DNA replication and inhibits the APC, so that Geminin (Gem) can accumulate and prevent relicensing of replication origins during S phase. (C) Endocycle oscillations in wild-type salivary glands predicted by computational modeling. Peaks of E2F1 protein were always followed by increases in CycE concentration that subsequently led to Geminin accumulation.

ent- and insulin-signaling-dependent kinase mTOR is active (Zielke et al. 2011). Moreover, E2F1 mRNA is selectively recruited to polyribosomes in the presence of growth signals. Thus, growth factor-dependent translation of E2F1 might couple cell growth to endoreplication.

The Rb–E2F pathway is also critical to placenta. The loss of Rb in TS cells results in up-regulation of the activator E2F3, which causes overproduction of trophoblast cells, disruption of placental architecture, and ultimately fetal death (Wenzel et al. 2007; Chong et al. 2009). The same phenotype results from ablation of the repressors E2F7 and E2F8 in trophoblast cell lineages (Ouseph et al. 2012). Notably, cells lacking both E2F3 activator and E2F7/E2F8 repressors undergo fairly normal endocycles (Ouseph et al. 2012), a phenomenon previously reported in flies (Frolov et al. 2001). Altogether, these data indicate that the Rb–E2F pathway is also essential for execution of endocycles. One specific target of this regulation may be the transcription factor p73 β . E2F1 up-regulates p73 β , which, in turn, activates transcription of the CDK-specific inhibitor p57 (Blint et al. 2002; Ma and Cress 2007) that is essential for initiating endoreplication in TS cells (discussed below).

Suppressing DNA Rereplication during Endocycles

DNA rereplication during mitotic cell cycles causes aneuploidy and genomic instability that leads to cancer (Storchova and Pellman 2004). However, because polyploid cells do not proliferate, the consequences of DNA rereplication in endoreplicating tissues are less severe. Nevertheless, with the exception of a few underreplicated regions, the fly genome is duplicated precisely during each endocycle (Nordman et al. 2011; Sher et al. 2012). Endocycling cells use the same mechanisms as proliferating cells to avoid premature initiation events, but several changes are required to compensate for the absence of mitosis.

In mitotic cell cycles, prereplication complexes (preRCs) are assembled at DNA replication origins distributed throughout the genome (Bell and Kaguni 2012). Restricting genome duplication to once per cell division requires that cells do not reinitiate nuclear DNA replication within regions that have already replicated until cell division is completed. Hence, preRC assembly occurs only when CDK activity is

suppressed during the anaphase to G₁-phase transition, and preRC activation occurs only when CDK and DDK activities are up-regulated during the G₁ to S-phase transition (Bell and Kaguni 2012). To prevent DNA rereplication, both flies and mammals use CDKs and ubiquitin ligases to inactivate one or more preRC proteins.

The anaphase promoting complex (APC) is a ubiquitin ligase that regulates the transition from mitosis to G₁ phase in eukarya (Pesin and Orr-Weaver 2008). It contains one of two activator proteins, either Fzr/Cdc20 or Frz/Cdh1, both of which target cyclin A, cyclin B, and Geminin in multicellular eukarya. Geminin prevents DNA rereplication, a role that is shared by CyclinA-Cdk2 (Zhu and Depamphilis 2009). APC^{Fzr/Cdc20}, which operates when CDK activity is high, triggers exit from metaphase into anaphase. APC^{Frz/Cdh1}, which operates when CDK activity is low, maintains G₁ phase by suppressing CDK activity and the expression of Geminin.

Fzr/Cdh1 is essential for endocycles in both *Drosophila* and mice (Sigrist and Lehner 1997; Schaeffer et al. 2004; García-Higuera et al. 2008), although not in *Arabidopsis* (Roodbarkelari et al. 2010). APC^{Fzr} activity oscillates during *Drosophila* endocycles (Narbonne-Reveau et al. 2008; Zielke et al. 2008) because CycE-Cdk2 phosphorylates and inactivates Fzr (Sigrist and Lehner 1997; Reber et al. 2006). The same appears to be true in mammals (Keck et al. 2007). Thus, low CycE-Cdk2 activity allows high APC^{Fzr/Cdh1} activity, which, in turn, degrades mitotic cyclins and Geminin, thereby allowing assembly of preRCs. Conversely, high CycE-Cdk2 activity inactivates APC^{Fzr/Cdh1}, thereby allowing the onset of S phase in the presence of Geminin (Fig. 2). Thus, Geminin protein levels oscillate in endoreplicating salivary glands as they do in proliferating cells. Moreover, this oscillation appears critical for endocycle progression, because constitutive expression of Geminin protein in salivary glands inhibits endocycles (Zielke et al. 2008). Nevertheless, Geminin is not essential for the development of salivary glands (Zielke et al. 2011), although it is essential for cell proliferation (Quinn et al.

N. Zielke et al.

Figure 2. Sustaining endocycles in mouse TG cells. Oscillation of APC activity and the levels of CDK-specific inhibitors and Geminin (Gmnn) are inversely related to oscillation of cyclin E (broken red line). APC activity and CDK inhibitor levels are high in G phases but low in S phases, whereas cyclin E is low in G phases but high in S phases. CcnE-Cdk2 activity is required to begin S phase. Endocycles result from a sequence of feedback loops, resulting from phosphorylation events by CcnE-Cdk2 and ubiquitination events by CRL1 and the APC. Ubiquitination by CRL1 requires CDK-dependent phosphorylation of its substrate. These events inhibit the activity of their protein targets and cause them to be degraded by the 26S proteasome (→). Protein symbols are those for mammals.

2001), suggesting that other mechanisms can prevent DNA rereplication in endocycling cells.

Suppressing CDK Activity during G Phase

During mitotic cell cycles, preRCs are assembled in the absence of CDK activity and activated in the presence of CDK activity (Bell and Kaguni 2012). This transition is facilitated by oscillations in the CIP/KIP family of cyclin-dependent kinase inhibitors (CKIs), which antagonize CycE-Cdk2 activity, as well as by the oscillation of APC activity. The same appears to be true for some endocycling cells as well.

Drosophila has only one CKI, “Dacapo” (Dap). Dap protein oscillations closely follow those of CycE in endoreplicating nurse cells of the ovary, and CycE promotes the accumulation of Dap RNA and protein (de Nooij et al. 2000). In the absence of Dap, nurse cells have reduced levels of the chromatin-bound MCM2–7 complex, S phase is extended and DNA damage accumulates, consistent with reduced preRC assembly during G phase (Hong et al. 2007). Dap, however, is not essential for endoreplication in flies. Mutation of *dap* in the germline allowed fairly normal nurse cell endoreplication (Hong et al. 2003), and endoreplication defects

were not detected in *dap* mutant salivary glands (Zielke et al. 2011). Moreover, Dap protein was not detected in endoreplicating follicle cells, salivary glands, or socket and shaft cells of the bristle lineage (Shcherbata et al. 2004; Audibert et al. 2005; Zielke et al. 2011). Hence, Dap appears to promote replication licensing during *Drosophila* endocycles by reinforcing low CDK activity during G phase in some cell types, but not in others. Where it is used, its activity appears to extend the window for DNA licensing, thereby increasing the number of active replication origins. This hypothesis would account for the fact that late-replicating heterochromatic sequences in *Drosophila* are underrepresented in some polyploid tissues (Nordman et al. 2011; Sher et al. 2012), but in *dap*^{-/-} nurse cells where CycE-Cdk2 activity is extended, these sequences are nearly fully replicated (Hong et al. 2007).

Mammals contain three CKIs that target Cdk1 and Cdk2 during cell proliferation and differentiation (Besson et al. 2008). CKIs p27^{Kip1} and p57^{Kip2} are structurally related proteins that share a conserved CDK-binding domain that targets them for CDK-dependent phosphorylation and ubiquitin-dependent degradation during S phase. However, their phenotypes are distinctly different. The mammalian ortholog of Dacapo, p27^{Kip1}, prevents premature entrance into S phase during mitotic cell cycles. Thus, p27^{-/-} mice are viable but show hyperproliferative characteristics expected for an inhibitor of cell proliferation. However, p57^{-/-} mice suffer developmental defects in multiple tissues leading to neonatal death (Zhang et al. 1997, and references therein). Remarkably, most of the developmental defects characteristic of p57^{-/-} mice can be corrected by expressing p27 protein from the p57 promoter, suggesting that p27 can substitute for p57 when necessary (Susaki et al. 2009). The p21^{Cip1} protein participates in DNA-damage-induced arrest of cell proliferation in G₁ and G₂ phases. Mice lacking p21 are viable and fertile, but sensitive to radiation.

Both p57 and p21 are up-regulated in TG cells, but only p57 levels oscillate during endocycles. Whereas p57 mRNA is expressed constitutively in TG cells, the p57 protein accumulates during the G phase of endocycles and is de-

graded during S phase (Fig. 2) (Hattori et al. 2000; Ullah et al. 2008). Degradation is triggered by CcnA-Cdk2-dependent phosphorylation of p57, which targets it for ubiquitination by CRL1-Skp2 followed by degradation by the 26S proteasome (Hattori et al. 2000; Kamura et al. 2003). Ectopic expression of a stable variant of p57 blocks DNA synthesis in TG cells (Hattori et al. 2000), indicating that the oscillation of p57 is crucial for endocycle progression. The fact that endocycles occur in p57^{-/-} TG cells when Cdk1 activity is selectively inhibited by RO3306 (Ullah et al. 2008) suggests that p27 is sufficient to sustain endocycles in the absence of p57. TS cells that lack p57 continue to proliferate in the absence of FGF4 until they become multinucleated TG cells (Ullah et al. 2008), which would account for the observation that trophoblasts in p57^{-/-} mice proliferate more extensively and still produce polyploid TG cells (Zhang et al. 1998).

The Role of ORC

The origin recognition complex (ORC) is a heterohexamer that is essential for origin licensing in eukaryotic cells (Bell and Kaguni 2012) and that is highly regulated during mitotic cell cycles in both flies and mammals (DePamphilis 2005). For example, DmOrc1 is degraded in an APC^{Fzr}-dependent manner and then resynthesized in response to E2F activation during late G₁ phase (Araki et al. 2005). Therefore, it is surprising that neither Orc1 nor Orc2 appeared to be essential for endoreplication in salivary glands (Park and Asano 2008). However, dramatic changes in the pattern of DNA replication in Orc2 mutants suggest that ORC is crucial for normal endoreplication and that the mild phenotype found in ORC mutants might be due to a small pool of ORCs that persists on the chromatin (Sher et al. 2012).

Cyclin A Plays a Role in Some *Drosophila* Endocycles

In contrast to mammals, where S phase is regulated by both cyclin A- and cyclin E-dependent Cdk2 activity, *Drosophila* CycA forms

N. Zielke et al.

complexes exclusively with Cdk1 (Knoblich et al. 1994) and is believed to function specifically in mitosis. Nevertheless, ectopic CycA can induce S phases in several *Drosophila* cell types, suggesting a possible S-phase function (Dong et al. 1997; Sprenger et al. 1997). Although the *cycA* and *cdk1* genes are not expressed in most *Drosophila* endocycles, CycA protein oscillates in endoreplicating cells of the mechanosensory bristle lineage, such that it is undetectable in G and early S phase, but accumulates during late S phase (Salle et al. 2012). Either increasing or decreasing the level of CycA reduces ploidy, delays S phase, and dramatically affects association of Orc2 with heterochromatic regions. This suggests that CycA-Cdk1 can regulate Orc2 localization and thereby modulate replication timing in endoreplicating bristle cells. Whether or not these effects of CycA during late S phase are limited to the bristle cell lineage remains to be determined. Expression of the mitotic regulators Cdk1, Stg/Cdc25, and CycA are decreased >1000-fold in endoreplicating salivary glands relative to mitotic cells, and these genes are therefore unlikely to regulate these endocycles (Zielke et al. 2008; Maqbool et al. 2010).

INITIATING ENDOREPLICATION

Flies

In the ovarian follicle cells and other endoreplicating tissues of *Drosophila*, the down-regulation of Cdk1/cdc2 is essential for the switch from mitotic cycling to endoreplication (Fig. 3). Indeed, this seems to be a universal requirement, because even fission yeast cells can be forced to undergo endocycles by mutating the p34/cdc2-p56/cdc13 complex (Hayles et al. 1994). Similarly, loss of *cdc2* (Cdk1) function in mitotically proliferating *Drosophila* cells can dramatically increase their DNA content (Hayashi 1996; Weigmann et al. 1997), although it was never determined whether these enlarged cells result from endocycles with distinct G phases or are produced via a more aberrant form of continuous rereplication.

A key upstream regulator of the mitotic-to-endocycle transition in ovarian follicle cells is

the Notch signaling pathway (Deng et al. 2001; Lopez-Schier and St Johnston 2001). Oocytes express the ligand Delta, which activates the Notch receptor in follicle cells. This results in Dacapo and String/Cdc25 being repressed, while the APC activator Fzr/Cdh1 is up-regulated (Schaeffer et al. 2004; Shcherbata et al. 2004). String/Cdc25 is a protein phosphatase that removes inhibitory phosphates from Cdk1, allowing it to associate with CycA and CycB and initiate mitosis. Thus, repression of String prevents mitosis. Up-regulating Fzr allows APC^{Fzr} to degrade CycA, CycB, thereby reinforcing the block to mitosis. Concomitant suppression of the CDK inhibitor Dacapo releases CycE/Cdk2, which is required to initiate S phase. Thus, in response to Notch signaling, follicle cells arrest in G₂ phase, assemble preRCs and then activate the preRCs when CycE-Cdk2 activity reaches the level needed to initiate endoreplication.

Notch signaling in the follicle cells activates the transcription factor *Hindsight* (*Hnt*), which represses String/Cdc25 and the transcription factor *Cut* (Sun and Deng 2007). Down-regulation of *Cut* allows Fzr/Cdh1 to accumulate. Thus, the switch to endoreplication occurs in two steps: First, Hnt-mediated down-regulation of String/Cdc25 arrests the cell in G₂ phase, and then down-regulation of *Cut* and subsequent derepression of Fzr/Cdh1 allow the cell to bypass mitosis and enter a G₁-like state that allows PreRC formation (Sun and Deng 2007). Interestingly, Delta/Notch signaling also promotes the switch from mitotic to endocycles during differentiation of enterocytes in the adult *Drosophila* intestine (J Xiang and BA Edgar, unpubl.), and thus this mechanism of developmental control may be widely used.

Some interesting tissue-specific differences exist. For example, the mitotic cyclins in endoreplicating 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). Furthermore, suppression of mitotic regulators in endoreplicating salivary glands is mediated, at least in part, by the transcriptional repressor E2F2 (Zielke et al. 2011).

Figure 3. Initiation of endoreplication in *Drosophila* follicle cells. (A) The follicle cells that encapsulate the ovary originate from stem cells and undergo three different cell cycle variants during their differentiation. Up to stage 6, follicle cells undergo normal mitotic divisions resulting in a final number of approximately 650 follicle cells. During stages 7–10A, follicle cells execute three endocycles. At stage 10B, follicle cells switch from endoreplication to gene amplification, in which specific loci become amplified by repeated origin activation. (B) At stages 6–7, up-regulation of the Notch ligand Delta (DI) in germline cells activates the Notch pathway in follicle cells, which results in inhibition of Stg/Cdc25 and activation of the transcription factor Hnt. Activation of Hnt prevents the transcriptional repressor Cut from inhibiting APC^{Fzr}, thereby promoting proteasomal degradation of mitotic cyclins and Stg/Cdc25. Down-regulation of Stg/Cdc25 arrests the cell in G₂ phase, whereas the subsequent degradation of mitotic cyclins suppresses the initiation of mitosis.

Initiation of endoreplication not only involves changes in the expression of cell cycle genes, but it also affects the response to DNA damage. Promoters of pro-apoptotic genes are silenced in endoreplicating cells, rendering them insensitive to genotoxic stress caused, for exam-

ple, by overexpression of Dup/Cdt1 (Mehrotra et al. 2008). This modification might be attributed to the fact that *Drosophila* polyploid cells include underreplicated regions containing stalled replication forks that would otherwise trigger a DNA damage response.

N. Zielke et al.

Mammals

Endoreplication in TS cells is triggered by depletion of FGF4 (Fig. 4). Deprivation of this mitogen rapidly induces expression of the p57^{Kip2} and p21^{Cip1} proteins in TS cells with a concomitant

loss of Cdk1 activity (Ullah et al. 2008). The decrease in Cdk1 activity could not be accounted for by corresponding changes in expression of either Cdk1 protein or cyclins A, B or E, suggesting that either p57 or p21 or both selectively inhibits Cdk1, but not Cdk2. If both Cdk2 and

Figure 4. Initiation of endoreplication in the mouse trophectoderm lineage. Chk1 is the effector kinase for two checkpoints. The “DNA replication checkpoint” prevents premature mitosis in response to DNA damage and stalled replication forks. These events activate (+) the ATR kinase that activates the Chk1 kinase that inhibits (⊥) the CDC25 phosphatase through site-specific phosphorylation. Because CDC25 is required to activate Cdk1, inhibition of CDC25 prevents entrance into mitosis. The “G₂ restriction point” prevents expression of p57 and p21 in response to mitogen stimulation. In the case of TS cells, the mitogen is FGF4. In the presence of FGF4, TS cells express the p21 and p57 genes, but Chk1 phosphorylates the p21 and p57 proteins, thereby targeting them for degradation (Ø) by the 26S proteasome. This prevents TS cells from exiting their mitotic cell cycle. In the absence of FGF4, Chk1 protein is degraded, thereby up-regulating p57 and p21. p21 may constitute a feedback loop that sustains suppression of Chk1 expression in TG cells (Gottifredi et al. 2001). Inhibition of Cdk1 by p57 triggers endoreduplication and differentiation into TG cells. p21 could facilitate this event by down-regulating Emi1, a specific inhibitor of the APC (Lee et al. 2009b). The APC targets mitotic cyclins and Geminin for degradation, thereby promoting origin licensing. The absence of Chk1 in TG cells allows p57 protein levels to oscillate. As CcnE levels increase, Cdk2-CcnE will eventually phosphorylate p57 at a CDK-specific site, thereby targeting it for degradation.

Cdk1 were inhibited, then DNA replication could not occur, because Cdk2 and Cdk1 are the only CDKs that trigger S phase during either mitotic cell cycles or endocycles (Ullah et al. 2008). In fact, endoreplication can be initiated in proliferating TS cells, as in other metazoan cells, by selectively inhibiting Cdk1 activity with a chemical analog (Hochegger et al. 2007; Ullah et al. 2008; Ma et al. 2009). Selective inhibition of Cdk1 reversibly arrests cells in G2 phase without inducing endoreplication, whereas cells arrested in prometaphase endoreplicate, because loss of Cdk1 activity then activates the APC, which degrades targets Geminin, cyclin A, and cyclin B for degradation. TS cells are unique, however, in that selective inhibition of Cdk1 by this method induces terminal differentiation in TG cells, whereas the same treatment induces apoptosis in embryonic stem cells. Thus, cells that are developmentally programmed for terminal differentiation avoid the apoptosis associated with DNA replication stress. In TG cells, this function, as well as others (Fig. 4), appears to be played by p21 (Ullah et al. 2008).

Only p57 is essential for switching from mitotic cell cycles to endocycles in TS cells. Not only are $p21^{-/-}$ mice viable and fertile, but FGF4 deprivation of TS($p21^{-/-}$) cells triggers endoreplication. In contrast, p57-deficient mice suffer multiple developmental abnormalities, and FGF4 deprivation of TS($p57^{-/-}$) does not trigger endoreplication. Instead, TS($p57^{-/-}$) cells undergo several rounds of cell division, express TG-cell-specific genes, and eventually form multinucleated TG cells, the consequence of multiple mitosis in the absence of cytokinesis (Ullah et al. 2008). Therefore, p57 is not essential either for expression of TG-specific genes or for inhibition of cytokinesis, and endoreplication can be replaced by acytokinetic mitoses. Nevertheless, TS($p57^{-/-}$) cells are capable of endoreplication, because selective inhibition of Cdk1 in these cells induces endocycles. Suppressing either expression of the endogenous p57 gene in wild-type TG cells or expression of a recombinant Cdk1 protein restores mitosis and results in multinucleated cells (Ullah et al. 2011). The ability of TS($p57^{-/-}$) cells to overproduce multinucleated TG cells

could account for the placentomegaly and pre-eclampsia observed in p57-deficient mice and humans (Knox and Baker 2007).

What links FGF4 deprivation to expression of p57 and p21? Remarkably, this involves Checkpoint kinase-1 (Chk1), a protein well known for its role in arresting cell proliferation in response to DNA damage or stalled replication forks. Although p21, p27, and p57 are transcribed and translated in TS cells, Chk1 phosphorylates p21 and p57 at specific sites, thereby selectively targeting them for degradation by the 26S proteasome (Ullah et al. 2011). When TS cells are deprived of FGF4, expression of Chk1 is suppressed. This allows unphosphorylated p57 and p21 proteins to accumulate and trigger endoreplication. The absence of Chk1 in TG cells also allows p57 levels to oscillate in response to changes in CcnE-Cdk2 activity. Ectopic expression of Chk1 in endocycling TG cells suppresses expression of p57 during G phases, thereby restoring mitosis, but not cytokinesis. Thus, in addition to its role in the DNA damage checkpoint, Chk1 serves as a mitogen-dependent kinase that prevents proliferating TS cells from exiting the mitotic cell cycle by premature expression of p57. The mechanism by which Chk1 expression is suppressed in response to FGF4 deprivation of TS cells remains to be elucidated, although it likely involves one of the ubiquitin ligases that target Chk1 for proteasomal degradation (Leung-Pineda et al. 2009; Zhang et al. 2009).

The function of Chk1 in TS cells is the equivalent of a “G₂ restriction point” (Fig. 4), in that Chk1 responds to changes in mitogenic stimuli by regulating cell proliferation. This dual role for Chk1 in sensing both DNA replication stress and mitogenic stress would account for the fact that Chk1 is essential for mammalian cell proliferation (Tang et al. 2006, and references therein). Because the ability of Chk1 to prevent p57 expression is not restricted to TS cells (Ullah et al. 2011), the “G₂ restriction point” presumably exists in all cells that are developmentally programmed for terminal differentiation by up-regulating either p57 or p21.

Suppression of Geminin activity also has been suggested as a mechanism for inducing

N. Zielke et al.

differentiation of pluripotent blastomeres into polyploid trophoblast cells, because *Gmnn*^{-/-} embryos overreplicate their DNA at the 8-cell stage and express trophoblast-specific genes (Gonzalez et al. 2006). Moreover, Geminin knockdown in mouse embryonal carcinoma and embryonic stem cells results in loss of stem cell identity and expression of trophoblast genes (Yang et al. 2011). However, others report that *Gmnn*^{-/-} embryos undergo DNA rereplication and apoptosis at the 8-cell stage (Hara et al. 2006) and that Geminin knockdown does not affect the ability of embryonic stem (ES) cells to maintain or exit pluripotency, although it does impair their ability to acquire a neural fate (Yellajoshiyula et al. 2011). Given that the overall level of Geminin remains constant during differentiation of TS cells into TG cells (Ullah et al. 2008) and that Geminin oscillates during endocycles in *Drosophila* cells as it does during mitotic cell cycles (Zielke et al. 2008), the simplest explanation is that Geminin prevents DNA rereplication during endocycles, as it does during mitotic cell cycles. In addition, Geminin may affect gene expression during preimplantation development as it does during postimplantation development (Yellajoshiyula et al. 2011).

CONCLUSION

The control mechanisms that govern endocycle progression in mammals, insects, and even plants share much in common, but given that all of these endocycles use the same machinery used to control S phase in mitotic cell cycles, this should not come as a surprise. However, distinct differences in regulatory modes—for instance, the use of CKIs—are also apparent between cell types and organisms. Although further research is required to determine how fundamental these differences really are, it seems likely that endocycles have arisen multiple times during evolution and therefore will show substantial diversity.

ACKNOWLEDGMENTS

We thank Terry Orr-Weaver and Michel Gho for helpful comments. Furthermore, we apologize

to the authors whose work could not be included due to space restrictions. The research in the Edgar Laboratory is supported by the DKFZ and the National Institutes of Health (grant GM51186). M. DePamphilis thanks the NICHD/NIH intramural program for funding.

REFERENCES

*Reference is also in this collection.

- Araki M, Yu H, Asano M. 2005. A novel motif governs APC-dependent degradation of *Drosophila* ORC1 in vivo. *Genes Dev* **19**: 2458–2465.
- Arias EE, Walter JC. 2006. PCNA functions as a molecular platform to trigger Cdt1 destruction and prevent re-replication. *Nat Cell Biol* **8**: 84–90.
- Audibert A, Simon F, Gho M. 2005. Cell cycle diversity involves differential regulation of Cyclin E activity in the *Drosophila* bristle cell lineage. *Development* **132**: 2287–2297.
- * Bell SP, Kaguni JM. 2012. Helicase loading at chromosomal origins of replication. *Cold Spring Harb Perspect Biol* doi: 10.1101/cshperspect.a010124.
- Besson A, Dowdy SE, Roberts JM. 2008. CDK inhibitors: Cell cycle regulators and beyond. *Dev Cell* **14**: 159–169.
- Blint E, Phillips AC, Kozlov S, Stewart CL, Vousden KH. 2002. Induction of p57^{KIP2} expression by p73β. *Proc Natl Acad Sci* **99**: 3529–3534.
- Bluteau D, Lordier L, Di Stefano A, Chang Y, Raslova H, Debili N, Vainchenker W. 2009. Regulation of megakaryocyte maturation and platelet formation. *J Thromb Haemost* **7**: 227–234.
- Britton JS, Edgar BA. 1998. Environmental control of the cell cycle in *Drosophila*: Nutrition activates mitotic and endoreplicative cells by distinct mechanisms. *Development* **125**: 2149–2158.
- Chong JL, Tsai SY, Sharma N, Opavsky R, Price R, Wu L, Fernandez SA, Leone G. 2009. E2f3a and E2f3b contribute to the control of cell proliferation and mouse development. *Mol Cell Biol* **29**: 414–424.
- * de la Paz Sanchez M, Costas C, Sequeira-Mendes J, Gutierrez C. 2012. DNA replication control in plants. *Cold Spring Harb Perspect Biol* doi: 10.1101/cshperspect.a010140.
- Deng WM, Althausen C, Ruohola-Baker H. 2001. Notch–Delta signaling induces a transition from mitotic cell cycle to endocycle in *Drosophila* follicle cells. *Development* **128**: 4737–4746.
- de Nooij JC, Graber KH, Hariharan IK. 2000. Expression of the cyclin-dependent kinase inhibitor Dacapo is regulated by cyclin E. *Mech Dev* **97**: 73–83.
- DePamphilis ML. 2005. Cell cycle dependent regulation of the origin recognition complex. *Cell Cycle* **4**: 70–79.
- Dong X, Zavitz KH, Thomas BJ, Lin M, Campbell S, Zipursky SL. 1997. Control of G₁ in the developing *Drosophila* eye: Rca1 regulates Cyclin A. *Genes Dev* **11**: 94–105.
- Edgar BA, Nijhout HF. 2004. Growth and cell cycle control in *Drosophila*. In *Cell growth—Control of cell size* (ed.

- Hall MN, et al.), pp. 23–83. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Edgar BA, Orr-Weaver TL. 2001. Endoreplication cell cycles: More for less. *Cell* **105**: 297–306.
- Eliades A, Papadantonakis N, Ravid K. 2010. New roles for cyclin E in megakaryocytic polyploidization. *J Biol Chem* **285**: 18909–18917.
- Follette PJ, Duronio RJ, O'Farrell PH. 1998. Fluctuations in cyclin E levels are required for multiple rounds of endocycle S phase in *Drosophila*. *Curr Biol* **8**: 235–238.
- Fox DT, Gall JG, Spradling AC. 2010. Error-prone polyploid mitosis during normal *Drosophila* development. *Genes Dev* **24**: 2294–2302.
- Frolov MV, Huen DS, Stevaux O, Dimova D, Balczarek-Strang K, Elsdon M, Dyson NJ. 2001. Functional antagonism between E2F family members. *Genes Dev* **15**: 2146–2160.
- García-Higuera I, Manchado E, Dubus P, Cañamero M, Méndez J, Moreno S, Malumbres M. 2008. Genomic stability and tumour suppression by the APC/C cofactor Cdh1. *Nat Cell Biol* **10**: 802–811.
- Geng Y, Yu Q, Sicinska E, Das M, Schneider JE, Bhattacharya S, Rideout WM, Bronson RT, Gardner H, Sicinski P. 2003. Cyclin E ablation in the mouse. *Cell* **114**: 431–443.
- Gonzalez MA, Tachibana KE, Adams DJ, van der Weyden L, Hemberger M, Coleman N, Bradley A, Laskey RA. 2006. Geminin is essential to prevent endoreduplication and to form pluripotent cells during mammalian development. *Genes Dev* **20**: 1880–1884.
- Gottifredi V, Karni-Schmidt O, Shieh SS, Prives C. 2001. p53 down-regulates CHK1 through p21 and the retinoblastoma protein. *Mol Cell Biol* **21**: 1066–1076.
- Hammond MP, Laird CD. 1985a. Chromosome structure and DNA replication in nurse and follicle cells of *Drosophila melanogaster*. *Chromosoma* **91**: 267–278.
- Hammond MP, Laird CD. 1985b. Control of DNA replication and spatial distribution of defined DNA sequences in salivary gland cells of *Drosophila melanogaster*. *Chromosoma* **91**: 279–286.
- Hara K, Nakayama KI, Nakayama K. 2006. Geminin is essential for the development of preimplantation mouse embryos. *Genes Cells* **11**: 1281–1293.
- Hattori N, Davies TC, Anson-Cartwright L, Cross JC. 2000. Periodic expression of the cyclin-dependent kinase inhibitor p57^{Kip2} in trophoblast giant cells defines a G₂-like gap phase of the endocycle. *Mol Biol Cell* **11**: 1037–1045.
- Havens CG, Walter JC. 2009. Docking of a specialized PIP Box onto chromatin-bound PCNA creates a degron for the ubiquitin ligase CRL4Cdt2. *Mol Cell* **35**: 93–104.
- Hayashi S. 1996. A Cdc2 dependent checkpoint maintains diploidy in *Drosophila*. *Development* **122**: 1051–1058.
- Hayles J, Fisher D, Woollard A, Nurse P. 1994. Temporal order of S phase and mitosis in fission yeast is determined by the state of the p34cdc2–mitotic B cyclin complex. *Cell* **78**: 813–822.
- Hochegger H, Dejsuphong D, Sonoda E, Saberi A, Rajendra E, Kirk J, Hunt T, Takeda S. 2007. An essential role for Cdk1 in S phase control is revealed via chemical genetics in vertebrate cells. *J Cell Biol* **178**: 257–268.
- Hong A, Lee-Kong S, Iida T, Sugimura I, Lilly MA. 2003. The p27^{cip/kip} ortholog *dacapo* maintains the *Drosophila* oocyte in prophase of meiosis I. *Development* **130**: 1235–1242.
- Hong A, Narbonne-Reveau K, Riesgo-Escovar J, Fu H, Aladjem MI, Lilly MA. 2007. The cyclin-dependent kinase inhibitor *Dacapo* promotes replication licensing during *Drosophila* endocycles. *EMBO J* **26**: 2071–2082.
- Hu D, Cross JC. 2010. Development and function of trophoblast giant cells in the rodent placenta. *Int J Dev Biol* **54**: 341–354.
- Kamura T, Hara T, Kotoshiba S, Yada M, Ishida N, Imaki H, Hatakeyama S, Nakayama K, Nakayama KI. 2003. Degradation of p57^{Kip2} mediated by SCF^{Skp2}-dependent ubiquitylation. *Proc Natl Acad Sci* **100**: 10231–10236.
- Keck JM, Summers MK, Tedesco D, Ekholm-Reed S, Chuang LC, Jackson PK, Reed SI. 2007. Cyclin E overexpression impairs progression through mitosis by inhibiting APC(Cdh1). *J Cell Biol* **178**: 371–385.
- Knoblich JA, Sauer K, Jones L, Richardson H, Saint R, Lehner CF. 1994. Cyclin E controls S phase progression and its down-regulation during *Drosophila* embryogenesis is required for the arrest of cell proliferation. *Cell* **77**: 107–120.
- Knox KS, Baker JC. 2007. Genome-wide expression profiling of placentas in the p57^{Kip2} model of pre-eclampsia. *Mol Hum Reprod* **13**: 251–263.
- Koepp DM, Schaefer LK, Ye X, Keyomarsi K, Chu C, Harper JW, Elledge SJ. 2001. Phosphorylation-dependent ubiquitination of cyclin E by the SCFF^{bw7} ubiquitin ligase. *Science* **294**: 173–177.
- Lee HO, Davidson JM, Duronio RJ. 2009a. Endoreplication: Polyploidy with purpose. *Genes Dev* **23**: 2461–2477.
- Lee J, Kim JA, Barbier V, Fotadar A, Fotadar R. 2009b. DNA damage triggers p21/WAF1-dependent Emi1 down-regulation that maintains G₂ arrest. *Mol Biol Cell* **20**: 1891–1902.
- Lee J, Kim JA, Margolis RL, Fotadar R. 2010. Substrate degradation by the anaphase promoting complex occurs during mitotic slippage. *Cell Cycle* **9**: 1792–1801.
- Leung-Pineda V, Huh J, Piwnicka-Worms H. 2009. DDB1 targets Chk1 to the Cul4 E3 ligase complex in normal cycling cells and in cells experiencing replication stress. *Cancer Res* **69**: 2630–2637.
- Lilly MA, Spradling AC. 1996. The *Drosophila* endocycle is controlled by Cyclin E and lacks a checkpoint ensuring S-phase completion. *Genes Dev* **10**: 2514–2526.
- Lopez-Schier H, St Johnston D. 2001. Delta signaling from the germ line controls the proliferation and differentiation of the somatic follicle cells during *Drosophila* oogenesis. *Genes Dev* **15**: 1393–1405.
- Ma Y, Cress WD. 2007. Transcriptional upregulation of p57^{Kip2} by the cyclin-dependent kinase inhibitor BMS-387032 is E2F dependent and serves as a negative feedback loop limiting cytotoxicity. *Oncogene* **26**: 3532–3540.
- Ma HT, Tsang YH, Marxer M, Poon RY. 2009. Cyclin A2-cyclin-dependent kinase 2 cooperates with the PLK1-SCF^β-TrCP1-EMI1-anaphase-promoting complex/cyclosome axis to promote genome reduplication in the absence of mitosis. *Mol Cell Biol* **29**: 6500–6514.

N. Zielke et al.

- Maqbool SB, Mehrotra S, Kolpakas A, Durden C, Zhang B, Zhong H, Calvi BR. 2010. Dampened activity of E2F1-DP and Myb-MuvB transcription factors in *Drosophila* endocycling cells. *J Cell Sci* **123**: 4095–4106.
- Mehrotra S, Maqbool SB, Kolpakas A, Murnen K, Calvi BR. 2008. Endocycling cells do not apoptose in response to DNA rereplication genotoxic stress. *Genes Dev* **22**: 3158–3171.
- Moberg KH, Bell DW, Wahrer DC, Haber DA, Hariharan IK. 2001. Archipelago regulates Cyclin E levels in *Drosophila* and is mutated in human cancer cell lines. *Nature* **413**: 311–316.
- Narbonne-Reveau K, Senger S, Pal M, Herr A, Richardson HE, Asano M, Deak P, Lilly MA. 2008. APC/CFzr/Cdh1 promotes cell cycle progression during the *Drosophila* endocycle. *Development* **135**: 1451–1461.
- Nordman J, Li S, Eng T, Macalpine D, Orr-Weaver TL. 2011. Developmental control of the DNA replication and transcription programs. *Genome Res* **21**: 175–181.
- Oda M, Shiota K, Tanaka S. 2010. Trophoblast cell lineage in cloned mouse embryos. *Dev Growth Differ* **52**: 285–291.
- Ouseph MM, Li J, Chen HZ, Pecot T, Wenzel P, Thompson JC, Comstock G, Chokshi V, Byrne M, Forde B, et al. 2012. Atypical E2F repressors and activators coordinate placental development. *Dev Cell* **22**: 849–862.
- Parisi T, Beck AR, Rougier N, McNeil T, Lucian L, Werb Z, Amati B. 2003. Cyclins E1 and E2 are required for endoreplication in placental trophoblast giant cells. *EMBO J* **22**: 4794–4803.
- Park SY, Asano M. 2008. The origin recognition complex is dispensable for endoreplication in *Drosophila*. *Proc Natl Acad Sci* **105**: 12343–12348.
- Pesin JA, Orr-Weaver TL. 2008. Regulation of APC/C activators in mitosis and meiosis. *Annu Rev Cell Dev Biol* **24**: 475–499.
- Pierce SB, Yost C, Britton JS, Loo LW, Flynn EM, Edgar BA, Eisenman RN. 2004. dMyc is required for larval growth and endoreplication in *Drosophila*. *Development* **131**: 2317–2327.
- Quinn LM, Herr A, McGarry TJ, Richardson H. 2001. The *Drosophila* Geminin homolog: Roles for Geminin in limiting DNA replication, in anaphase and in neurogenesis. *Genes Dev* **15**: 2741–2754.
- Reber A, Lehner CF, Jacobs HW. 2006. Terminal mitoses require negative regulation of Fzr/Cdh1 by Cyclin A, preventing premature degradation of mitotic cyclins and String/Cdc25. *Development* **133**: 3201–3211.
- Roodbarkelari F, Bramsiepe J, Weinl C, Marquardt S, Novak B, Jakoby MJ, Lechner E, Genschik P, Schnittger A. 2010. Cullin 4-ring finger-ligase plays a key role in the control of endoreplication cycles in *Arabidopsis* trichomes. *Proc Natl Acad Sci* **107**: 15275–15280.
- Salle J, Campbell SD, Gho M, Audibert A. 2012. CycA is involved in the control of endoreplication dynamics in the *Drosophila* bristle lineage. *Development* **139**: 547–557.
- Schaeffer V, Althausen C, Shcherbata HR, Deng WM, Ruohola-Baker H. 2004. Notch-dependent Fizzy-related/Hec1/Cdh1 expression is required for the mitotic-to-endocycle transition in *Drosophila* follicle cells. *Curr Biol* **14**: 630–636.
- Shcherbata HR, Althausen C, Findley SD, Ruohola-Baker H. 2004. The mitotic-to-endocycle switch in *Drosophila* follicle cells is executed by Notch-dependent regulation of G₁/S, G₂/M and M/G₁ cell-cycle transitions. *Development* **131**: 3169–3181.
- Sher N, Bell GW, Li S, Nordman J, Eng T, Eaton ML, Macalpine DM, Orr-Weaver TL. 2012. Developmental control of gene copy number by repression of replication initiation and fork progression. *Genome Res* **22**: 64–75.
- Shibutani S, Swanhart LM, Duronio RJ. 2007. Rbf1-independent termination of E2f1-target gene expression during early *Drosophila* embryogenesis. *Development* **134**: 467–478.
- Shibutani ST, de la Cruz AF, Tran V, Turbyfill WJ 3rd, Reis T, Edgar BA, Duronio RJ. 2008. Intrinsic negative cell cycle regulation provided by PIP box- and Cul4Cdt2-mediated destruction of E2f1 during S phase. *Dev Cell* **15**: 890–900.
- Sigrist SJ, Lehner CF. 1997. *Drosophila* fizzy-related down-regulates mitotic cyclins and is required for cell proliferation arrest and entry into endocycles. *Cell* **90**: 671–681.
- Smith AV, Orr-Weaver TL. 1991. The regulation of the cell cycle during *Drosophila* embryogenesis: The transition to polyteny. *Development* **112**: 997–1008.
- Sprenger F, Yakubovich N, O'Farrell PH. 1997. S-phase function of *Drosophila* cyclin A and its downregulation in G₁ phase. *Curr Biol* **7**: 488–499.
- Storchova Z, Pellman D. 2004. From polyploidy to aneuploidy, genome instability and cancer. *Nat Rev Mol Cell Biol* **5**: 45–54.
- Strohmaier H, Spruck CH, Kaiser P, Won KA, Sangfelt O, Reed SI. 2001. Human F-box protein hCdc4 targets cyclin E for proteolysis and is mutated in a breast cancer cell line. *Nature* **413**: 316–322.
- Sun J, Deng WM. 2007. Hindsight mediates the role of notch in suppressing Hedgehog signaling and cell proliferation. *Dev Cell* **12**: 431–442.
- Susaki E, Nakayama K, Yamasaki L, Nakayama KI. 2009. Common and specific roles of the related CDK inhibitors p27 and p57 revealed by a knock-in mouse model. *Proc Natl Acad Sci* **106**: 5192–5197.
- Szuplewski S, Sandmann T, Hietakangas V, Cohen SM. 2009. *Drosophila* Minus is required for cell proliferation and influences Cyclin E turnover. *Genes Dev* **23**: 1998–2003.
- * Tanaka S, Araki H. 2012. Formation of pre-initiation complex: Formation of the active helicase and establishment of replication forks. *Cold Spring Harb Perspect Biol* doi: 10.1101/cshperspect.a010371.
- Tang J, Erikson RL, Liu X. 2006. Checkpoint kinase 1 (Chk1) is required for mitotic progression through negative regulation of Polo-like kinase 1 (Plk1). *Proc Natl Acad Sci* **103**: 11964–11969.
- Tetzlaff MT, Yu W, Li M, Zhang P, Finegold M, Mahon K, Harper JW, Schwartz RJ, Elledge SJ. 2004. Defective cardiovascular development and elevated cyclin E and Notch proteins in mice lacking the Fbw7 F-box protein. *Proc Natl Acad Sci* **101**: 3338–3345.
- Ullah Z, Kohn MJ, Yagi R, Vassilev LT, DePamphilis ML. 2008. Differentiation of trophoblast stem cells into giant cells is triggered by p57/Kip2 inhibition of CDK1 activity. *Genes Dev* **22**: 3024–3036.

- Ullah Z, Lee CY, Depamphilis ML. 2009. Cip/Kip cyclin-dependent protein kinase inhibitors and the road to polyploidy. *Cell Div* **4**: 10.
- Ullah Z, de Renty C, DePamphilis ML. 2011. Checkpoint kinase 1 prevents cell cycle exit linked to terminal cell differentiation. *Mol Cell Biol* **31**: 4129–4143.
- Unhavaithaya Y, Orr-Weaver TL. 2012. Polyploidization of glia in neural development links tissue growth to blood–brain barrier integrity. *Genes Dev* **26**: 31–36.
- van den Heuvel S, Dyson NJ. 2008. Conserved functions of the pRB and E2F families. *Nat Rev Mol Cell Biol* **9**: 713–724.
- Weigmann K, Cohen SM, Lehner CF. 1997. Cell cycle progression, growth and patterning in imaginal discs despite inhibition of cell division after inactivation of *Drosophila* Cdc2 kinase. *Development* **124**: 3555–3563.
- Weingartner M, Criqui MC, Meszaros T, Binarova P, Schmit AC, Helfer A, Derevier A, Erhardt M, Bogre L, Genschik P. 2004. Expression of a nondegradable cyclin B1 affects plant development and leads to endomitosis by inhibiting the formation of a phragmoplast. *Plant Cell* **16**: 643–657.
- Weiss A, Herzig A, Jacobs H, Lehner CF. 1998. Continuous Cyclin E expression inhibits progression through endoreduplication cycles in *Drosophila*. *Curr Biol* **8**: 239–242.
- Weng L, Zhu C, Xu J, Du W. 2003. Critical role of active repression by E2F and Rb proteins in endoreplication during *Drosophila* development. *EMBO J* **22**: 3865–3875.
- Wenzel PL, Wu L, de Bruin A, Chong JL, Chen WY, Dureska G, Sites E, Pan T, Sharma A, Huang K, et al. 2007. Rb is critical in a mammalian tissue stem cell population. *Genes Dev* **21**: 85–97.
- Yang VS, Carter SA, Hyland SJ, Tachibana-Konwalski K, Laskey RA, Gonzalez MA. 2011. Geminin escapes degradation in G₁ of mouse pluripotent cells and mediates the expression of Oct4, Sox2, and Nanog. *Curr Biol* **21**: 692–699.
- Yellajoshyula D, Patterson ES, Elitt MS, Kroll KL. 2011. Geminin promotes neural fate acquisition of embryonic stem cells by maintaining chromatin in an accessible and hyperacetylated state. *Proc Natl Acad Sci* **108**: 3294–3299.
- Zhang P, Liegeois NJ, Wong C, Finegold M, Hou H, Thompson JC, Silverman A, Harper JW, DePinho RA, Elledge SJ. 1997. Altered cell differentiation and proliferation in mice lacking p57^{KIP2} indicates a role in Beckwith-Wiedemann syndrome. *Nature* **387**: 151–158.
- Zhang P, Wong C, DePinho RA, Harper JW, Elledge SJ. 1998. Cooperation between the Cdk inhibitors p27^{KIP1} and p57^{KIP2} in the control of tissue growth and development. *Genes Dev* **12**: 3162–3167.
- Zhang YW, Brognard J, Coughlin C, You Z, Dolled-Filhart M, Aslanian A, Manning G, Abraham RT, Hunter T. 2009. The F box protein Fbx6 regulates Chk1 stability and cellular sensitivity to replication stress. *Mol Cell* **35**: 442–453.
- Zhu W, Depamphilis ML. 2009. Selective killing of cancer cells by suppression of geminin activity. *Cancer Res* **69**: 4870–4877.
- Zielke N, Querings S, Rottig C, Lehner C, Sprenger F. 2008. The anaphase-promoting complex/cyclosome (APC/C) is required for rereplication control in endoreplication cycles. *Genes Dev* **22**: 1690–1703.
- Zielke N, Kim KJ, Tran V, Shibutani ST, Bravo MJ, Nagarajan S, van Straaten M, Woods B, von Dassow G, Rottig C, et al. 2011. Control of *Drosophila* endocycles by E2F and CRL4(CDT2). *Nature* **480**: 123–127.

Cold Spring Harbor Perspectives in Biology

Endoreplication

Norman Zielke, Bruce A. Edgar and Melvin L. DePamphilis

Cold Spring Harb Perspect Biol 2013; doi: 10.1101/cshperspect.a012948

Subject Collection [DNA Replication](#)

Replication of Epstein–Barr Viral DNA

Wolfgang Hammerschmidt and Bill Sugden

Replication Proteins and Human Disease

*Andrew P. Jackson, Ronald A. Laskey and
Nicholas Coleman*

Break-Induced DNA Replication

*Ranjith P. Anand, Susan T. Lovett and James E.
Haber*

Regulating DNA Replication in Eukarya

Khalid Siddiqui, Kin Fan On and John F.X. Diffley

Archaeology of Eukaryotic DNA Replication

Kira S. Makarova and Eugene V. Koonin

Translesion DNA Polymerases

Myron F. Goodman and Roger Woodgate

**Human Papillomavirus Infections: Warts or
Cancer?**

Louise T. Chow and Thomas R. Broker

Chromatin and DNA Replication

David M. MacAlpine and Geneviève Almouzni

Endoreplication

*Norman Zielke, Bruce A. Edgar and Melvin L.
DePamphilis*

Replication-Fork Dynamics

*Karl E. Duderstadt, Rodrigo Reyes-Lamothe,
Antoine M. van Oijen, et al.*

**Helicase Activation and Establishment of
Replication Forks at Chromosomal Origins of
Replication**

Seiji Tanaka and Hiroyuki Araki

Poxvirus DNA Replication

Bernard Moss

**The Minichromosome Maintenance Replicative
Helicase**

Stephen D. Bell and Michael R. Botchan

DNA Replication Origins

Alan C. Leonard and Marcel Méchali

**Principles and Concepts of DNA Replication in
Bacteria, Archaea, and Eukarya**

*Michael O'Donnell, Lance Langston and Bruce
Stillman*

DNA Replication Timing

Nicholas Rhind and David M. Gilbert

For additional articles in this collection, see <http://cshperspectives.cshlp.org/cgi/collection/>

**All Modifications and
Oligo Types Synthesized**
Long Oligos • Fluorescent • Chimeric • DNA • RNA • Antisense

Oligo Modifications?
Your wish is our command.