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Endoreplication Cell Cycles: More for Less

Review

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Molecular studies from the past decade have revealed striking conservation in the mechanisms of eukaryotic cell cycle control. Yet before the advent of molecular genetics, it was clear that eukaryotes possessed many different cell cycle variations, and thus that there must be diversity in mechanisms of control. One common cell cycle variant is the endoreplication cycle, in which cells increase their genomic DNA content without dividing. Although endocycles are sometimes dismissed as an evolutionary peculiarity, they are widespread in protists, plants, and many animals including arthropods, mollusks, and mammals. Endocycling cells can become incredibly polyploid, with chromatin values (C values denote DNA content as a multiple of the normal haploid genome) as high as 24,000 reported in some plant endosperms (Traas et al., 1998). Because cell size for a given cell type is generally proportional to the amount of nuclear DNA, endoreplication constitutes an effective strategy of cell growth, and it is often found in differentiated cells that are large or highly metabolically active (see Figure 1 for a classic example). Recent studies show that endocycles utilize much of the same G1/S regulatory machinery as mitotic cycles, with some informative, and some perplexing, alterations. Here we review what is known about the different endocycle types found in nature and how they are regulated. We also offer some ideas about why endocycles are so commonly used.

Definitions

Endoreplication, also known as endoreduplication, gives rise to cells with extra copies of the genomic DNA. In many cases the chromosome number is increased in multiples of N (the normal haploid chromosome number). Such cells are referred to as polyploid or endopolyploid (Figure 2A). Endoreplicated cells in which the sister chromatids remain closely associated are referred to as polytene (Figure 2B). Thus whereas sperm nuclei are 1N, 1C, diploid cells in G1 are 2N, 2C, and diploid cells in G2 are 2N, 4C, a polyploid cell might be 32N, 32C or 32N, 64C. Polytene cells are normally descended from diploid progenitors, but since homologs are paired, the equivalent polytene cell would be 1N, 64C (White, 1973). The best known example of polyteny is found in the giant salivary gland chromosomes of *Drosophila*, which have up to 2048 copies of the euchromatic genome neatly aligned in parallel arrays (see Urata et al., 1995), but polytene chromosomes greater than 16,000C have been noted in another insect, *Chironomus*. The distinction between polyteny and polyploidy is not absolute, and various intermediate DNA configurations can occur, differing primarily in the degree of association between duplicated chromatids (Figure 2D, Hammond and Laird, 1985). For this reason the term *polyploid* is often used to refer to endoreplicated cells with virtually any chromosomal configuration.

In endoreplication cell cycles, or endocycles, S phases alternate with distinct gap phases that lack DNA replication, but there is no cell division (Figure 3). Few, if any, cases of polypoidy resulting from continuous DNA replication have been reported. Some endocycling cell types retain hallmarks of mitosis, but many examples lack all vestiges of mitosis, including chromosome condensation, nuclear envelope breakdown, and the reorganization of microtubules that builds the spindle. The term endomitosis initially referred to a rare cell cycle in which mitosis occurred without nuclear envelope breakdown or cytokinesis (for review see Nagl, 1978). However, now this term is more generally used to describe cycles that proceed through anaphase but lack nuclear division and cytokinesis. In yet another cell cycle variant, nuclear division occurs without cytokinesis, giving rise to multinucleate cells. Such cycles are seen in mammalian hepatocytes and osteoclasts, and also in syncytial slime molds like *Physarum* and early insect embryos. The regulation of these cycles is thought to be similar to that found in normal mitotic cells, and is not covered here.

Regulatory Mechanisms

In proliferating cells, phosphorylation events triggered by cyclin-dependent kinases (CDKs) control the onset of both mitosis and S phase. Mitotic control is mediated by A and B type cyclins complexed with Cdk1, and also by Cdc25 type phosphatases that serve to activate Cdk1. S phase initiation and progression are mediated by Cyclin E/Cdk2 and Cyclin A/Cdk2 complexes, whereas earlier cell cycle events including G0→G1 transitions involve the D type cyclins complexed with Cdks 4 and 6. Endoreplicating cells appear to have simplified this regulatory machinery by eliminating the expression of components that are no longer required. For instance, some endoreplicating cell types that bypass mitosis do not express Cdk1 or its activators, Cyclin B, Cyclin A, and Cdc25C. Cell types in which mitosis is partially traversed but incomplete have been reported to have lowered levels of these G2/M regulators. This suggests that levels of mitotic CDK activity may be responsible for determining the extent of mitotic functions retained in an endocycling cell. However, some studies have found that endocycling cells can also lose structural components required for mitosis, such as centrosomes (Mahowald et al., 1979). Although the loss of structural mitotic components is a plausible mechanism for initiating endocycling, it could also be a secondary event with little functional relevance.

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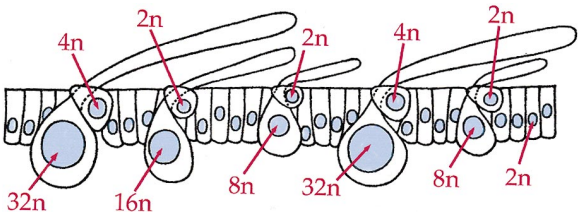

Figure 1. Cell Size Is Correlated with Ploidy

An example from the wing epithelium of the moth *Ephestia.* Scale producing cells endoreplicate up to 32N, and the size of both the cell bodies and scales (apical protrusions) is proportional to their ploidy. Nuclei are filled in blue, and N values denoted in red. (Adapted from: Alfred Kühn, *Vorlesungen über entwicklungsphysiologie*, Springer-Verlag, Berlin, 1955.)

What enables endocycling cells to replicate their DNA many times within a single interphase, and thus escape the mechanisms that block rereplication in mitotic cells? This is not entirely clear yet, but work in diverse mitotic systems does suggest what sort of mechanisms to expect. Many recent studies indicate that after completion of an S phase, cyclin/CDK activity must drop to low levels before rereplication is possible. A window of low CDK activity is initiated by destruction of the G2 cyclins during late metaphase by the anaphase promoting complex (APC), which remains active through early G1. Low CDK activity during early G1 allows the assembly of prereplication complexes (preRCs) containing ORC proteins, Cdc6, Cdt1/Dup1, and MCMs onto DNA replication origins, thus "licensing" the DNA for another round of replication. Cdc6 and the MCM proteins are removed from the DNA during and after S phase, and their ability to rebind and relicense the DNA for replication is inhibited during the S and G2 phases by high levels of Cdk2 and Cdk1 activity. It is only after levels of Cdk activity drop during the M→G1 transition that relicensing becomes possible again. In this way only a single round of DNA replication is allowed in each mitotic cycle (see Donaldson and Blow, 1999, for review).

Other switches that are built into this CDK↔APC tug-of-war may further ensure against inappropriate rereplication. For instance CDK inhibitors such as Sic1 in yeast and $p27^{Kip1}$ and $p57^{Kip2}$ in vertebrates can be targeted for turnover by CDK-dependent phosphorylation, and Geminin, a protein that sequesters Cdt1/Dup1 and thus inhibits replication licensing, is periodically degraded at anaphase, probably by the APC (Tada et al., 2001; Wohlschlegel et al., 2000). In endoreplicating cells, these mechanisms could in principle confer intrinsic oscillatory capability upon the S phase kinase, CycE/Cdk2, and thus also upon the machinery that first assembles and then "fires" (activates) preRCs. Oscillations in Cyclin E activity and MCM localization (Su and O'Farrell, 1998) have been reported in endocycles, and the Geminin target Cdt1/Dup1 also appears to be used (Whittaker et al., 2000). Moreover, the fact that all endocycles exhibit gap phases suggests that, as in mitotic cycles, CDK activity must periodically cease to allow reassembly of preRCs and renewed DNA replication. Indeed, enforcing continuous CycE/Cdk2 activity causes endocycles to stall in *Drosophila* (Follette et al., 1998; Weiss et al., 1998). Below we describe the different types of endocycles characterized in the literature and note how each fits with these general notions of S phase control.

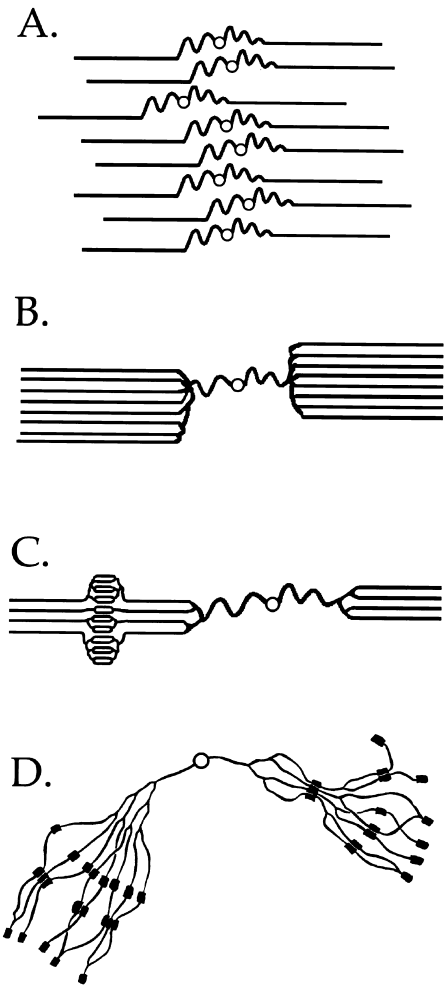

Figure 2. Chromosome Configurations Produced by Different Types of Endocycles

(A) Separated sister chromatids in an 8N polyploid cell; (B) aligned polytene chromosomes in which the centric heterochromatin (wavy line) is underreplicated; (C) polytene chromosomes with an amplified region; and (D) proposed chromosome structure in a polytene nurse cell from *Drosophila*, illustrating that the extent of replication and sister chromatid pairing (black boxes) vary regionally (after Hammond and Laird, 1985). Centromeres are indicated with open circles in all panels.

Endocycling in Mammals

Megakaryocytes are a blood cell type specialized to produce platelets. As part of their differentiation program, megakaryocytes become polyploid up to 128N (for review, see Zimmet and Ravid, 2000). This conversion to polyploidy is achieved via endomitosis, and is triggered by the secreted signal thrombopoietin. The large increase in megakaryocyte size that results from polyploidy is correlated with their ability to bud off adequate numbers of platelets. Cyclin D3 protein is upregulated in response to thrombopoietin in these cells, and Cyclin D3 overexpression in the megakaryocyte lineage has been found to increase megakaryocyte ploidy in

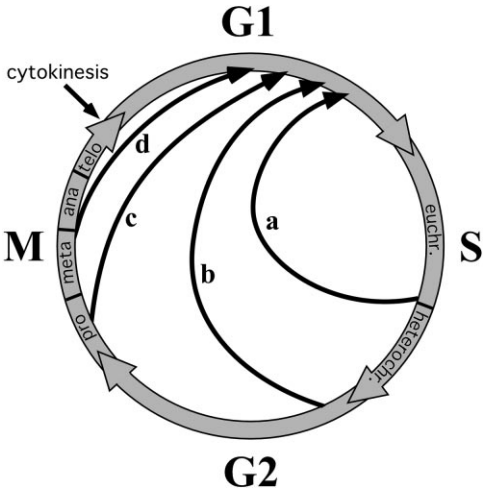

Figure 3. Endocycles Bypass Aspects of S Phase and Mitosis to Different Extents

The gray circle indicates a generic mitotic cell cycle, and the black arrows indicate truncated cell cycles found in different types of endoreplicating cells.

a: *Drosophila* larval tissues, post-cycle 6 ovarian nurse cells.

b: Cycle 1–4 *Drosophila* nurse cells.

c: Cycle 5 *Drosophila* nurse cells, mammalian giant trophoblasts.

d: Endomitosis in mammalian megakaryocytes.

transgenic mice (Zimmet et al., 1997). Antisense experiments with megakaryocyte cell lines show that decreasing the levels of Cyclin D3 blocks polyploidization, and thus Cyclin D3 appears to be critical for driving thrombopoietin-induced endocycles (Wang et al., 1995). Studies of *Drosophila* Cyclin D/Cdk4 show that this complex can also increase ploidy in endocycling tissues, and suggest that it does this by potentiating cellular growth, rather than via direct effects on cell cycle regulators such as RBF, a fly *retinoblastoma* homolog (Datar et al., 2000). Knockout studies in mice are also consistent with the idea that Cdk4 and the D-type cyclins are used to regulate growth, but this possibility has not yet been tested extensively. In the case of megakaryocyte endocycling, it is not yet known what the critical targets of the Cyclin D3 kinase complex are.

Endomitosis in megakaryocytes involves nuclear envelope breakdown and the appearance of condensed chromosomes and multipolar spindles (Nagata et al., 1997). Sister chromatids have been observed to separate in anaphase A, but anaphase B does not occur. As a consequence, replicated copies of the chromosomes are incorporated into the same nucleus when the nuclear envelope reforms at a stage equivalent to telophase. Cytokinesis appears to be bypassed completely. Megakaryocyte endomitosis is reported to occur at subnormal levels of the mitotic regulatory kinase, Cyclin B/Cdk1 (reviewed in Zimmet and Ravid, 2000), and the observation has been made that degradation of Cyclin B is relatively enhanced (Zhang et al., 1998). Thus, premature or excess degradation of Cyclin B might account for the exit from mitosis prior to anaphase B, nuclear division, and cytokinesis.

Mammalian trophoblasts, which contribute to the placenta, also become polyploid via endocycles. These cells increase their DNA content to >1000C, possibly to facilitate the high metabolic activity required of them (reviewed in Zybina and Zybina, 1996). The chromosomes of trophoblast giant cells have regions in which replicated copies are tightly associated (Varmuza et al., 1988). Thus their chromosomes are polytene, although they are not aligned along their entire lengths to produce the intricate banding patterns seen in insect polytene cells. DNA replication in trophoblasts is cyclic, and aspects of mitosis are present. The chromosomes bundle in what has been termed "endoprophase," and then decondense and dissociate in "endointerphase" when DNA replication occurs. Changes in cell cycle regulators were investigated in the trophoblast cell line, Rcho1 (MacAuley et al., 1998). During Rcho1 polytenization, Cyclin D1 is induced, Cyclins E and A remain high, and levels of Cyclin B are reduced. The CDK inhibitor (CKI) $p57^{Kip2}$ is upregulated as Rcho1 cells differentiate and become polytene (Hattori et al., 2000). p57 protein accumulates at the end of each S phase and disappears several hours before each subsequent S phase. Furthermore, a mutation that stabilizes p57 blocks S phase entry, suggesting that this inhibitor may mediate fluctuations in Cyclin E/Cdk2 activity and thus allow periodic replication origin licensing and firing. Interestingly, the mutation used to stabilize p57 encompasses a putative CDK phosphorylation site. This suggests that there may be feedback between CDK activity and p57 stability, and so provides one of the switches required to build a biphasic oscillator. As described below, a CDK/CKI-based oscillator may also be a plausible explanation for how some endocycles are controlled in *Drosophila*.

An interesting parallel exists between the control of differentiation and polytenization of trophoblasts and the maintenance of diploidy during *Drosophila* development. The *Drosophila* Escargot protein, a member of the *snail* family of transcription factors, is required to prevent diploid abdominal histoblasts from becoming polyploid during larval development, when the majority of the tissues are in the endocycle (Fuse et al., 1994; Hayashi, 1996; Hayashi et al., 1993). A mouse gene (*mSna*) that is most homologous to Escargot is downregulated during trophoblast differentiation and polytenization (Nakayama et al., 1998). Overexpression of mSna in rat Rcho1 cells reduced the frequency with which these cells differentiated to form giant polytene trophoblast cells, but did not affect the kinetics of endocycles in trophoblasts that had already differentiated. Both the *Drosophila* Escargot and mSna proteins are expressed in a variety of diploid tissues. These observations have led to the proposal that the Escargot and mSna transcription factors affect differentiation decisions that preclude endoreplication.

Endocycling in Plants

Many plants contain tissues that become highly polyploid during development. A survey of *Arabidopsis* revealed polyploidy in hair trichomes, leaf epidermal cells, root tip cells, and cells in the hypocotyl (Galbraith et al., 1991; Melaragno et al., 1993). In some cell types the extent of endoreplication appears to be intrinsically controlled by the differentiation program, but environmental influences such as light can also affect endoreplication

(see Traas et al., 1998 for review). Examples of somatic polyploidy are particularly prevalent in plants with small genome sizes, raising the possibility that high ploidy may be needed for certain aspects of plant growth or function (De Rocher et al., 1990; Nagl, 1976). Proposals include a requirement for increased cell size to achieve a particular morphology, the need to change nuclear to organelle ratio, and the use of increased gene copy number to cope with environmental damage. Polyploidization in the maize endosperm has been proposed to increase its metabolic capability.

Cell cycle regulators have been analyzed in *Arabidopsis*, maize, and alfalfa endocycles. Regulators that control the G1-S transition in mammalian cells, particularly the Rb-Cyclin D pathway, are conserved in plants (for review see den Boer and Murray, 2000). During polyploidization of maize endosperm, mitotic cyclin/Cdk activity has been reported to decrease, whereas S phase cyclin/Cdk is maintained. In this case, however, the specific cyclins and CDKs involved have not been defined (Grafi et al., 1996; Grafi and Larkins, 1995). Investigation of polyploidization in alfalfa demonstrated a novel role for proteolysis in the endocycle (Cebolla et al., 1999). An ortholog to the Cdh1/Fzr-related family of proteins, which activate the anaphase promoting complex and promote G2 cyclin degradation in other species, was isolated from alfalfa and called *ccs52*. Strikingly, the *ccs52* gene is expressed in endocycling cells, and when antisense RNA was used to reduce the level of *ccs52* transcripts, ploidy in these cells was reduced. There was no effect on diploid cells. This suggests that APC-mediated proteolysis may be a key event in generating polyploidy. As described above, periodic APC activation is one mechanism that might permit preRC assembly and licensing between S phases. It remains unclear which specific APC targets might be involved in endocycling in alfalfa, or whether APC-mediated proteolysis plays a role in other endocycles. Investigations in this area may shed considerable light on the mechanisms of endocycle progression.

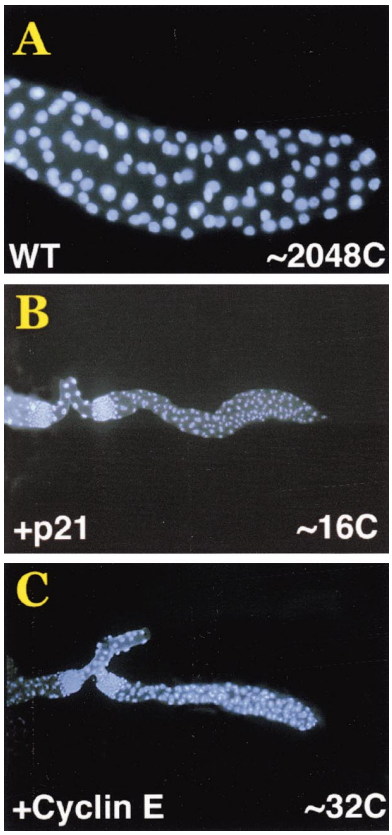

Figure 4. The Relationship of Growth and Endocycle Progression in the Polytene Salivary Glands of *Drosophila* Salivary Glands

All glands are from third instar animals of the same age and size. DNA is stained with DAPI (blue). Normally (A) the salivary cells polytenize to 2048C. When endocycle progression is stalled at ~16C by forced expression of the CDK inhibitor $p21^{CIP1}$ (B) the glands fail to grow. Endocycle progression can also be blocked by continuous overexpression of Cyclin E (C), suggesting that Cyclin E expression must be periodic for progression through successive endocycles. p21 or Cyclin E were expressed using the ptc-Gal4/UAS system. Photo courtesy of Jessica Britton.

Endocycling in Insects

Endoreplication is widespread in arthropods (White, 1973) and has been extensively characterized in the tiny but highly polyploid fruit fly, *D. melanogaster.* Following the cell-proliferative phase of *Drosophila* embryogenesis, many tissues initiate endocycles that lack all visible aspects of mitosis (Smith and Orr-Weaver, 1991). These tissues, which include the gut, epidermis, fat body (liver), malipighian tubules (kidney), trachea, and salivary glands, continue to endocycle during larval development long after they are fully differentiated. Some adult tissues, including ovarian follicle and nurse cells, and sensory neurons in the wing, also employ endocycles. Endoreplication parallels larval growth, and experimental inhibition of endocycle progression using DNA replication inhibitors, or mutations in genes essential for DNA replication, shows that increases in C values are required for both cell and organismal growth (Figure 4). Conversely, conditions that arrest growth invariably block endocycle progression, suggesting a tight regulatory linkage between these two processes (Galloni and Edgar, 1999; Zhang et al., 2000). Final DNA levels in the larval cells appear to be developmentally programmed, ranging from 16C to 2048C depending on cell type.

In switching from mitotic to endo cycles, cells in the *Drosophila* embryo terminate the expression of mRNAs encoding the mitotic regulators Cdk1, Cyclin A, Cyclin B, Cyclin B3, and Cdc25/*string* (Sauer et al., 1995). How transcription of these genes is shut off at this juncture is not known, but developmentally programmed signals are presumed to be important. In addition to preprogrammed transcriptional controls, embryonic cells switching to the endocycle downregulate their mitotic machinery at the protein level. Studies of Fzr/Cdh1, a regulator of the APC, show that APC activity is required for the switch from mitotic to endocycles. *fzr* mutant embryos show inappropriate accumulation of G2 cyclins in G1 cells that are poised to initiate endocycling, and these cells never initiate their first endocycle S phases (Sigrist and Lehner, 1997). This is presumed to be due to the inhibitory action of persistent APC targets such as Cyclin A, Cyclin B, or perhaps Geminin, on preRC formation. Consistent with this idea, experimental inhibi-

tion of Cdk1 activity in fission yeast, budding yeast, and *Drosophila* can force cells that are normally mitotic to become endoreplicative (Hayashi, 1996).

The possibility that APC-mediated proteolysis might be generally used in endocycle progression is suggested by the observation in alfalfa noted above (Cebolla et al., 1999), but it is still unclear whether Fzr or the APC functions during successive *Drosophila* endocycles. The expression pattern of Fzr suggests that the Fzr/APC may be required only for the initial switch from mitotic to endocycles, and that it is irrelevant thereafter. Analysis of the *Drosphila* mutant *morula*, however, suggests that this may not be the case in all cell types (Reed and Orr-Weaver, 1997). Weak alleles of *morula* exhibit the striking phenotype that polyploid nurse cells exit the endocycle and resume mitosis. This is accompanied by reappearance of Cyclin B protein via a posttranscriptional mechanism, suggesting that APC function may be compromised.

Of the examples described here, *Drosophila's* endocycles are the most distinct from the archetypal cell cycle. Not only do these cycles lack mitosis, but genomic replication during each S phase is incomplete. In most polytene tissues heterochromatin, which makes up approximately 30% of the genome, is underreplicated (Figures 2 and 3). This includes centromeric heterochromatin, intercalary (or interband) heterochromatin, and telomereic sequences. In addition, certain euchromatic regions, such as the histone genes, can be underreplicated (Laird 1980; Hammond and Laird, 1985). Heterochromatic replication is affected by levels of Cyclin E; in weak *cyclin E* mutants heterochromatin in the ovarian nurse cells is replicated (Lilly and Spradling, 1996). Although the mechanism by which Cyclin E affects heterochromatic DNA replication is unclear, the idea most consistent with available data is that lowered levels of Cyclin E allow S phase to continue for longer, and that this in turn allows heterochromatin (which is normally late replicating even in mitotic cells) to replicate (Lilly and Spradling, 1996). This might be because reduced levels of Cyclin E affect the oscillations in CDK activity that regulate preRC assembly and time S and Gap phases (see below and Figure 5).

Developmental changes in *Drosophila's* ovarian nurse cells have provided additional insights into the parameters of the endocycle (see Figures 2 and 3). During the first five endocycles of nurse cell development, the chromosomes are polytene, with tightly associated sister chromatids. In these cycles, genomic replication is complete and heterochromatin replicates late in S phase (Dej and Spradling, 1999). After the S phase of the fifth endocycle, the chromosomes condense and separate from each other. In subsequent cycles the chromosomes are polyploid in appearance, although an association between replicated chromatids is maintained (Dej and Spradling, 1999). In addition to this cytological change, heterochromatin and some euchromatic regions such as the histone genes are underreplicated from cycle 6 on (Figure 2D; Hammond and Laird, 1985). These observations reveal that the endocycle can retain some properties of prophase and that developmental controls can alter the extent of DNA replication. An even more extreme example of this sort of developmental switching has been reported in epidermal cells of *Manduca sexta* caterpillars. These cells endocycle to 32C and then, in response to a natural increase in the steroid hormone ecdysone, reenter mitotic cycles and reduce their ploidy back down to 2C (Kato et al., 1987).

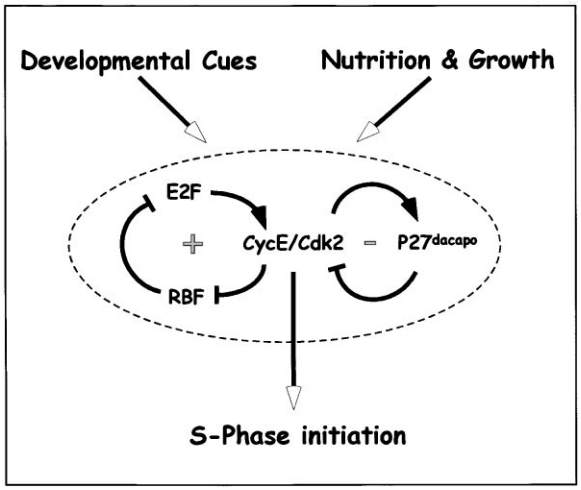

Figure 5. Hypothetical Feedback Relationships in the Gene Network that Regulates Endocycling in *Drosophila*

Both positive (+) and negative (-) loops are suggested. This might allow pulses of CycE/Cdk2 activity to be generated from continuous input signals derived from states of cell growth and determination.

Another alteration observed in polyploid insect cells is a relaxation of DNA replication controls to permit amplification of specific genomic regions (Figure 2C). In the polytene salivary glands of the fungus fly, *Sciara*, a genomic region undergoes amplification (Liang et al., 1993). The somatic follicle cells of the *Drosophila* ovary become polyploid, but after wholesale genomic replication ceases, four specific chromosomal intervals continue to endoreplicate (Calvi et al., 1998). The genes in these regions are thus amplified. Two of the amplified regions contain genes encoding eggshell chorion proteins whose production at high levels is required for normal eggshell formation. Chorion gene amplification provides an excellent metazoan model for replication origin usage in which regulatory elements and replication initiation sites have been defined. Binding of the origin recognition complex (ORC) and other initiation proteins has recently been demonstrated at these control elements (Austin et al., 1999; Whittaker et al., 2000). Interestingly, *Drosophila* E2F1 and the *retinoblastoma* homolog, RBF, also appear to be located in DNA-bound ORC complexes during DNA amplification (Bosco et al., 2001). In *rbf* mutants, the follicle cells fail to switch from genome-wide endoreplication to gene amplification correctly, and undergo an extra round of wholesale endoreplication (Bosco et al., 2001). In addition, an increased number of rounds of amplification occur. These observations suggest that RBF is important both for limiting DNA endoreplication during development and controlling origin firing, and that it may execute these functions directly upon the replication machinery.

Regulatory Switches in Endocycle Control

One central regulator of all *Drosophila* endocycles is the S phase kinase, Cyclin E/Cdk2. The *cyclin E* gene is

transcribed prior to the onset of endocycle S phases and is required for these cycles (Knoblich et al., 1994). The E2F1/DP transcription factor, thought to be important for Cyclin E transcription, is also required for normal endocycle progression (Royzman et al., 1997). Ectopic induction of Cyclin E or E2F1 can trigger precocious DNA replication in endoreplicating tissues, even after their cells have been forced into a quiescent state by nutrient deprivation (Britton and Edgar, 1998). It is generally believed that Cyclin E/Cdk2 is the trigger that "fires" preRCs by phosphorylating as yet unidentified components therein (Duronio et al., 1998). One recent study of endoreplicating salivary glands, however, found that Cyclin E induction caused the relocalization of an MCM protein to chromatin, and the authors suggested (somewhat provocatively) that Cyclin E might also be involved in preRC assembly (Su and O'Farrell, 1998; but see also Hua et al., 1997).

In apparent contrast to its role in promoting endocycling, continuous overexpression of Cyclin E inhibits endocycle progression in *Drosophila* (Follette et al., 1998; Weiss et al., 1998; Figure 4). This suggests that oscillations in Cyclin E activity may be required for successive endocycling. In fact, oscillations of Cyclin E protein have been documented in endocycling ovarian nurse cells (Lilly and Spradling, 1996), and *cyclin E* mRNA fluctuates in the endocycling embryonic gut (Duronio and O'Farrell, 1995) as well as in endocycling larval tissues. Although these fluctuations are believed to reflect S/G periodicity, this has been clearly documented so far only in a few embryonic endocycles. As noted above, fluctuations in Cyclin E levels have not been observed in endocycling mammalian trophoblasts (Hattori et al., 2000), but in this case the kinase activity of Cyclin E/Cdk2 may nevertheless fluctuate due to periodic expression of the CKI, $p57^{Kip2}$.

It has been suggested that Cyclin E/Cdk2 activity may have to drop between S phases to allow preRC assembly and relicensing of the DNA for replication (Hua et al., 1997; Follette et al., 1998; Weiss et al., 1998). Much experimental work from mitotic systems supports the idea that CDK activity inhibits preRC assembly, but two apparently contrary observations from flies warrant mention. First, all 16 mitotic cycles of *Drosophila* embryogenesis proceed in the presence of high, continuous Cyclin E/Cdk2 activity (Sauer et al., 1995). Second, although continuous overexpression of Cyclin E in mitotically proliferating wing imaginal cells does truncate G1 and elongate S phases, it fails to arrest cell cycle progression altogether. These apparent paradoxes suggest that preRC assembly can occur in mitotic *Drosophila* cells even in the presence of high Cyclin E activity. This might be explained by differences between mitotic and endocycle preRC components, and in fact one *Drosophila* MCM protein has been described that may be used in mitotic but not endocycle S phases (Feger et al., 1995). Another possibility is that nuclear envelope breakdown, which occurs in mitotic cycles but is bypassed in *Drosophila's* endocycles, may transiently lower nuclear levels of Cyclin E/Cdk2 and thus facilitate preRC assembly even while cytoplasmic Cdk2 activity remains high. In this regard it is interesting that Cyclin E is present but does not detectably oscillate during gene amplification in the ovarian follicle cells (Calvi et al., 1998). This suggests the existence of special "amplification factors," yet to be identified, that permit reinitiation of DNA synthesis at amplicons in the presence of Cyclin E.

What mechanisms account for the periodicity of Cyclin E in endocycling cells? Although there are clues, this central problem is still unresolved in any system. One possibility is that "hard wired" developmental programs explicitly specify the number of pulses of Cyclin E expression, and thus the number of S phases, that occur in each different endocycling cell type. Such preprogrammed cues orchestrate many aspects of cell cycle control during fly embryogenesis, including the patterning of mitoses, cell cycle exit into G1/G0, and the transition to endocycling. Transcriptional control of Cyclin E is known to be involved in the latter two transitions, and the underlying mechanism is presumed to be combinatorial control of transcription via the gene's very large, complex promotor region (Jones et al., 2000). The idea that the embryonic endocycles are preprogrammed is also supported by studies showing that these cycles are patterned just as are the preceding mitotic cycles (Smith and Orr-Weaver; 1991), and that the endocycles are initiated and patterned correctly even in *string* mutants, which sustain a G2 cell cycle arrest early in embryogenesis long before the transition to polyteny. The fly embryo, however, may be a special case. Stereotyped patterning of Cyclin E expression and DNA replication have not been reported for the many endocycles that occur later in *Drosophila* development, and most of these cycles appear to be asynchronous within tissues (Smith and Orr-Weaver 1991; Lilly and Spradling 1996). Moreover, most of the larval tissues that endoreplicate are fully differentiated and thus are expected to be insensitive to many types of developmental signaling. For instance, genetic manipulations of Notch, Hedgehog, FGF, DPP, and EGF signaling in these tissues have no effect on endocycle activation (Britton and Edgar, 1998). Instead, external influences such as nutrition and endocrine hormones regulate the larval endocycles to a large degree (see below).

One alternative to micromanagement by developmental programming posits that the gene network controlling endocycling acts as an oscillator, reading out pulses of Cyclin E expression in response to continuous inputs such as nutrition. Although this possibility has not yet been tested rigorously, the available data do indicate the existence of feedback controls that might confer periodicity upon Cyclin E. One of many plausible oscillators is shown in Figure 5. This particular mechanism is suggested by data obtained in several different developmental contexts, as follows. First, Cyclin E and the E2F1/DP transcription factor, both of which are required for normal endocycles (Knoblich et al., 1994; Royzman et al., 1997), can act as mutual activators of each other, and thus might cooperate in a positive feedback loop to initiate G→S transitions (Duronio et al., 1996). Observations parallel to findings in vertebrates show that *Drosophila* Cyclin E/Cdk2 can potentiate E2F1 activity by phosphorylating and inactivating the E2F1 corepressor RBF, whereas E2F1 can promote transcription of the *cyclin E* gene. Second, Cyclin E can also negatively regulate its own transcription, in endocycling embryonic cells (Sauer et al., 1995). The mechanism for this is obscure, but one possibility involves *dacapo*, a

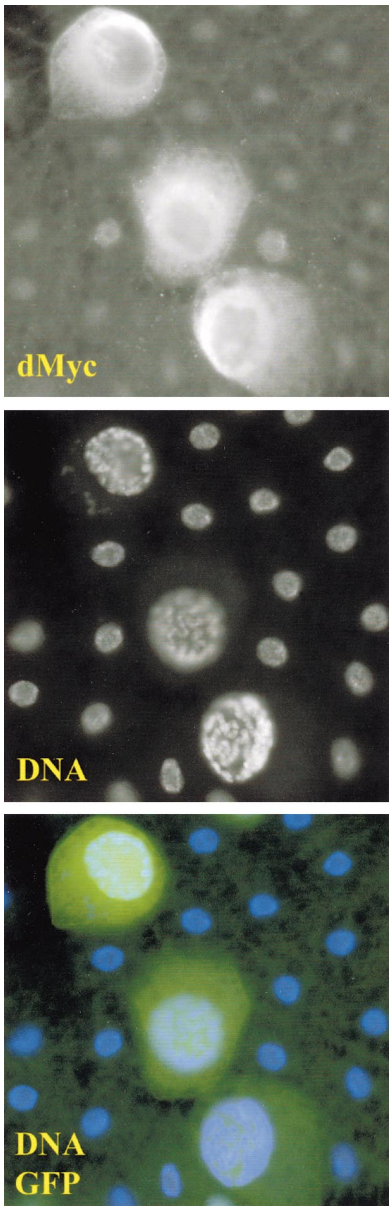

Figure 6. Experimentally Upregulating Cellular Growth Promotes Hyperendoreplication

In this example the transcription factor dMyc (Johnston et al., 1999; Britton, 2000) was clonally overexpressed in a subpopulation of cells in the fat body of *Drosophila* using the FLP/Act>>Gal4/UAS transgene system. GFP is coexpressed with dMyc, marking three large cells in the center of the photo. The surrounding GFP-negative cells are unaffected. Fat body cells normally endoreplicate uniformly to 256C, but the increased growth caused by excess dMyc allows these cells to endoreplicate to >2048C. Photo courtesy of Ling Li.

fly $p27^{Cip/Kip}$ homolog that specifically inhibits Cyclin E/Cdk2 activity. Dacapo protein oscillates in endocycling ovarian nurse cells, just out of phase with Cyclin E, and this oscillation is Cyclin E dependent. Moreover, overexpressed Cyclin E or E2F1 can increase the expression of *dacapo* in mitotic *Drosophila* cells (de Nooij et al., 2000). These relationships provide the ingredients for a negative feedback loop that might autoextinguish Cyclin E expression after S phase initiation, and perhaps also indirectly squelch E2F1 activity. Consequent loss of Cyclin E and E2F1 activity might then cause *dacapo* levels to decline, allowing Cyclin E activity to rise again and fire another S phase. Computer simulations suggest that such a flip-flop oscillator might work in principle (E. Meier and B. Edgar, unpublished), but establishing whether this or any sort of oscillator actually operates in vivo will require some very careful experimentation.

Lest the reader become too enamored with cell cycle oscillators, we caution that some of the data already contradict the specific feedback relationships shown in Figure 5. For instance, ovarian nurse cells lacking RBF—an essential component of the oscillator diagrammed in Figure 5—appear to support endocycles (Du and Dyson, 1999). The notion that positive feedback exists between Cyclin E and E2F1 is only weakly supported, being based principally on data from overexpression experiments that are not clearly consistent with loss-of-function mutant analysis (Duronio and O'Farrell, 1995; Sauer et al., 1995). Moreover, Cyclin E and E2F1 probably do not require each other for activity. Expression of E2F1 target genes occurs even in *cyclin E* mutants (Duronio and O'Farrell, 1995), whereas the regulatory region of *Drosophila's cyclin E* locus contains many modular enhancer elements and two promoters (Jones et al., 2000), only one of which is thought to be E2F responsive. Nonetheless, the idea that feedback regulation intrinsic to the cell cycle control apparatus confers periodicity on Cyclin E/Cdk2 activity is compelling since it would permit a periodic output—successive rounds of preRC assembly and firing—to be driven by continuous input signals. As described below, these upstream signals are most likely derived from states of cell specification and cell growth.

Upstream Regulators of the Endocycle: Differentiation and Growth

What are the upstream regulators of *Drosophila's* endocycles? As noted above, the levels of ploidy achieved by the various larval tissues appear to be developmentally preprogrammed, ranging from as little as 16C in some gut and epidermal cells to as much as 2048C in the famous polytene cells of the salivary glands. Genetically coding the number of cycles each cell type undergoes might seem the best way to achieve this, but in fact one can imagine many ways in which an oscillator might also be subject to cell type-specific regulation. For instance, relative levels the oscillator's components could be affected by cell type-specific factors, and this might affect the frequency of preRC firing, or the developmental window within which DNA replication was possible. One attractive scenario of this type involves direct coupling of the oscillator's frequency to metabolic rate, a cell type-specific characteristic. This idea is supported by several observations. First, progression of *Drosophila's* larval endocycles is tightly coupled to an ongoing influx of dietary protein from feeding (Britton and Edgar, 1998). These endocycles can be stopped, restarted, slowed, or accelerated by altering feeding regimes. Altering nutritional conditions, temperature, or the hormonal system that defines the feeding period also changes the final ploidy of the larval tissues. Second, numerous

genes required for protein synthesis and dTOR, a protein kinase thought to link nutritional signaling to the protein synthetic machinery, are required for endocycling (Galloni and Edgar, 1999; Zhang et al., 2000). Third, the nutritional requirement for endocycling can be bypassed by forced expression of factors that autonomously promote cellular growth such as the dMyc transcription factor, the Cyclin D/Cdk4 complex, the catalytic subunit of phosphoinositide 3-kinase, and the *Drosophila* insulin receptor (Johnston et al., 1999; Datar, 2000; Britton, 2000; Edgar et al., 2001). Each of these growth promoting activities can also promote hyperpolyploidy of endocycling tissues in well-fed animals. For instance the larval fat body, which normal endoreplicates to 256C, can be driven to >2000C by overexpression of dMyc (Figure 6). The effects of these factors on cell metabolism are not yet well defined in flies, but protein synthesis and nutrient uptake are likely targets (see Stocker and Hafen, 2000, for review). How cell growth rates might influence the S phase control apparatus in endoreplicating tissues is still unclear. Studies in mitotic systems suggest that growth rates and/or nutrition may influence the accumulation of the G1 cyclins Cln3, Cyclin D1, Cyclin D2, and Cyclin E at the protein level via translation or turnover (Polymenis and Schmidt, 1997; Muise-Helmericks et al., 1998; Perez-Roger et al., 1999; Prober and Edgar, 2000), but this possibility has not yet been tested in any endotissues.

Conclusions

So what is the utility of endocyles? Given their widespread occurrence, there are probably many correct answers to this question. Nevertheless some generalizations can be offered. One property intrinsic to many endocycles is that once they are initiated, further mitotic divisions are ill advised for mechanical reasons. Another consideration is that polyploidization allows cells to increase their mass or metabolic output. Finally, since endocycling permits growth without periodic rearrangements of cytoskeletal elements or cell-cell contacts, as happens in mitosis, it is less disruptive to highly structured tissues than is mitotic proliferation. Together these properties conspire to make the endocycle an advantageous strategy for cells and tissues that are terminally differentiated yet must continue to grow. In addition, polyploid cells are extremely resistant to conditions that induce DNA damage, probably because they have many copies of every gene, and because they need not segregate their chromosomes during mitosis. This might be quite advantageous for some cellular or organismal lifestyles. A number of substantial and intriguing questions remain. For example, what genetic programs mediate the switch from mitotic to endocycles? Is an intrinsic oscillator used for successive endocycling? How might continuous growth signals interface with such an oscillator? How frequently did novel mechanisms for endocycling arise during evolution, and how extreme are the variations in regulation? Answers to these questions promise to provide insights that pertain not only to endoreplication, but also to the mechanisms used in proliferative cycles and growth control.

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References

Austin, R.J., Orr-Weaver, T.L., and Bell, S.P. (1999). Drosophila ORC specifically binds to ACE3, an origin of DNA replication control element. Genes Dev. *13*, 2639–2649.

Bosco, G., Du, W., and Orr-Weaver, T.L. (2001). DNA replication control through interaction of E2F-RB and the origin recognition complex. Nat. Cell Biol. *3*, 289–295.

Britton, J.S. (2000). Genetic and environmental control of growth and the cell cycle during larval development of *Drosophila melanogaster*. Ph.D. Thesis, University of Washington, Seattle, WA.

Britton, J.S., and Edgar, B.A. (1998). Environmental control of the cell cycle in *Drosophila*: nutrition activates mitotic and endoreplicative cells by distinct mechanisms. Development *125*, 2149–2158.

Calvi, B.R., Lilly, M.A., and Spradling, A.C. (1998). Cell cycle control of chorion gene amplification. Genes Dev. *12*, 734–744.

Cebolla, A., Vinardell, J.M., Kiss, E., Olah, B., Roudier, F., Kondorosi, A., and Kondorosi, E. (1999). The mitotic inhibitor ccs52 is required for endoreduplication and ploidy-dependent cell enlargement in plants. EMBO J. *18*, 4476–4484.

Datar, S.A. (2000). Developmental regulation of growth and cell cycle progression in *Drosophila melanogaster*: a larval growth arrest screen, and molecular and genetic analysis of the Cyclin D Cdk4 complex. Ph.D. Thesis, University of Washington, Seattle, WA.

Datar, S.A., Jacobs, H.W., Lehner, C.F., and Edgar, B.A. (2000). The *Drosophila* cyclinD/Cdk4 complex promotes cellular growth. EMBO J. *19*, 4543–4554.

Dej, K.J., and Spradling, A.C. (1999). The endocycle controls nurse cell polytene chromosome structure during Drosophila oogenesis. Development *126*, 293–303.

den Boer, B.G., and Murray, J.A. (2000). Triggering the cell cycle in plants. Trends Cell Biol. *10*, 245–250.

de Nooij, J.C., Graber, K.H., and Hariharan, I.K. (2000). Expression of the cyclin-dependent kinase inhibitor Dacapo is regulated by Cyclin E. Mech. Dev. *97*, 73–83.

De Rocher, E.J., Harkins, K.R., Galbraith, D.W., and Bohnert, H.J. (1990). Developmentally regulated endopolyploidy in succulents with small genomes. Science *250*, 99–101.

Donaldson, A.D., and Blow, J.J. (1999). The regulation of replication origin activation. Curr. Opin. Genet. Dev. *9*, 62–68.

Du, W., and Dyson, N. (1999). The role of RBF in the introduction of G_1 regulation during *Drosophila* embryogenesis. EMBO J. *18*, 916–925.

Duronio, R.J., and O'Farrell, P.H. (1995). Developmental control of the G_1 to S transition in *Drosophila*: cyclin E is a limiting downstream target of E2F. Genes Dev. *9*, 1456–1468.

Duronio, R.J., Brook, A., Dyson, N., and O'Farrell, P.H. (1996). E2F-induced S phase requires *Cyclin E*. Genes Dev. *10*, 2505–2513.

Duronio, R.J., Bonnette, P.C., and O'Farrell, P.H. (1998). Mutations of the Drosophila dDP, dE2F, and cyclin E genes reveal distinct roles for the E2F-DP transcription factor and cyclin E during the S phase transition, Mol. Cell. Biol. *18*, 141–151.

Edgar, B.A., Britton, J., de la Cruz, A.F.A., Johnston, L.A., Lehman, D., Martin-Castellanos, C., and Prober, D. (2001) Pattern- and growth-linked cell cycles in *Drosophila* development. In The Cell Cycle and Development. John Wiley & Sons (Novartis Found. Symp. *237*, 3–18).

Feger, G., Vaessin, H., Su, T.T., Wolff, E., Jan, L.Y., and Jan, Y.N. (1995). dpa, a member of the MCM family, is required for mitotic DNA replication but not endoreplication in Drosophila. EMBO J. *14*, 5387–5398.

Follette, P.J., Duronio, R.J., and O'Farrell, P.H. (1998). Fluctuations in cyclin E levels are required for multiple rounds of endocycle S phase in Drosophila. Curr. Biol. *8*, 235–238.

Fuse, N., Hirose, S., and Hayashi, S. (1994). Diploidy of Drosophila imaginal cells is maintained by a transcriptional repressor encoded by escargot. Genes Dev. *8*, 2270–2281.

Galbraith, D.W., Harkins, K.R., and Knapp, S. (1991). Systemic endopolyploidy in *Arabidopsis thaliana*. Plant Physiol. *96*, 985–989.

Galloni, M., and Edgar, B.A. (1999). Cell-autonomous and non-autonomous growth-defective mutants of *Drosophila melanogaster*. Development *126*, 2365–2375.

Grafi, G., and Larkins, B.A. (1995). Endoreduplication in maize endosperm—involvement of m-phase-promoting factor inhibition and induction of s-phase-related kinases. Science *269*, 1262–1264.

Grafi, G., Burnett, R.J., Helentjaris, T., Larkins, B.A., DeCaprio, J.A., Sellers, W.R., and Kaelin, W.G., Jr. (1996). A maize cDNA encoding a member of the retinoblastoma protein family: involvement in endoreduplication. Proc. Natl. Acad. Sci. USA *93*, 8962–8967.

Hammond, M.P., and Laird, C.D. (1985). Chromosome structure and DNA replication in nurse and follicle cells of *Drosophila melanogaster*. Chromosoma *91*, 267–278.

Hattori, N., Davies, T.C., Anson-Cartwright, L., and Cross, J.C. (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.

Hayashi, S. (1996). A cdc2 dependent checkpoint maintains diploidy in drosophila. Development *122*, 1051–1058.

Hayashi, S., Hirose, S., Metcalfe, T., and Shirras, A.D. (1993). Control of imaginal cell development by the escargot gene of Drosophila. Development *118*, 105–115.

Hua, X.H., Yan, H., and Newport, J. (1997). A role for Cdk2 kinase in negatively regulating DNA replication during S phase of the cell cycle. J. Cell Biol. *137,* 183–192.

Johnston, L.A., Prober, D.A., Edgar, B.A., Eisenman, R.N., and Gallant, P. (1999). Drosophila *myc* regulates cellular growth during development. Cell *98*, 779–790.

Jones, L., Richardson, H., and Saint, R. (2000). Tissue-specific regulation of cyclin E transcription during *Drosophila melanogaster* embryogenesis. Development *127*, 4619–4630.

Kato, Y., Nair, K.K., Dyer, K.A., and Riddiford, L.M. (1987). Changes in ploidy level of epidermal cells during last larval instar of the tobacco hornworm, *Manduca* sexta. Development *99*, 137–143.

Knoblich, J.A., Sauer, K., Jones, L., Richardson, H., Saint, R., and Lehner, C.F. (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.

Laird, C.D. (1980). Structural paradox of polytene chromosomes. Cell *22*, 869–874.

Liang, C., Spitzer, J.D., Smith, H.S., and Gerbi, S.A. (1993). Replication initiates at a confined region during DNA amplification in Sciara DNA puff II/9A. Genes Dev. *7*, 1072–1084.

Lilly, M.A., and Spradling, A.C. (1996). The Drosophila endocycle is controlled by Cyclin E and lacks a checkpoint ensuring S-phase completion. Genes Dev. *10*, 2514–2526.

MacAuley, A., Cross, J.C., and Werb, Z. (1998). Reprogramming the cell cycle for endoreduplication in rodent trophoblast cells. Mol. Biol. Cell *9*, 795–807.

Mahowald, A.P., Caulton, J.H., Edwards, M.K., and Floyd, A.D. (1979). Loss of centrioles and polyploidization in follicle cells of Drosophila melanogaster. Exp. Cell Res. *118*, 404–410.

Melaragno, J.E., Mehrotra, B., and Coleman, A.W. (1993). Relationship between endopolyploidy and cell size in epidermal tissue of Arabidopsis. Plant Cell *5*, 1661–1668.

Muise-Helmericks, R.C., Grimes, H.L., Bellacosa, A., Malstrom, S.E., Tschilis, P.N., and Rosen, N. (1998). Cyclin D expression is controlled post-transcriptionally via a phosphatidylinositol 3-kinase/AKT-dependent pathway. J. Biol. Chem. *273*, 29864–29872.

Nagata, Y., Muro, Y., and Todokoro, K. (1997). Thrombopoietin-induced polyploidization of bone marrow megakaryocytes is due to a unique regulatory mechanism in late mitosis. J. Cell Biol. *139*, 449–457.

Nagl, W. (1976). DNA endoreduplication and polyteny understood as evolutionary strategies. Nature *261*, 614–615.

Nagl, W. (1978). Endopolyploidy and Polyteny in Differentiation and Evolution (Amsterdam: North-Holland Publishing Company).

Nakayama, H., Scott, I.C., and Cross, J.C. (1998). The transition to endoreduplication in trophoblast giant cells is regulated by the mSNA zinc finger transcription factor. Dev. Biol. *199*, 150–163.

Perez-Roger, I., Kim, S., Griffiths, B., Sewing, A., and Land, H. (1999). Cyclins D1 and D2 mediate Myc-induced proliferation via sequestration of p27kip1 and p21cip1. EMBO J. *18*, 5310–5320.

Polymenis, M., and Schmidt, E.V. (1997). Coupling of cell division to cell growth by translational control of the G_1 cyclin CLN3 in yeast. Genes Dev. *11*, 2522–2531.

Prober, D.A., and Edgar, B.A. (2000). Ras promotes cellular growth in the *Drosophila* wing. Cell *100*, 435–446.

Reed, B.H., and Orr-Weaver, T.L. (1997). The Drosophila gene morula inhibits mitotic functions in the endo cell cycle and the mitotic cell cycle. Development *124*, 3543–3553.

Royzman, I., Whittaker, A.J., and Orr-Weaver, T.L. (1997). Mutations in *Drosophila DP* and *E2F* distinguish G1-S progression from an associated transcriptional program.

Royzman, I., Austin, R.J., Bosco, G., Bell, S.P., and Orr-Weaver, T.L. (1999). ORC localization in Drosophila follicle cells and the effects of mutations in dE2F and dDP. Genes Dev. *13*, 827–840.

Sauer, K., Knoblich, J.A., Richardson, H., and Lehner, C.F. (1995). Distinct modes of cyclin E/cdc2c kinase regulation and S-phase control in mitotic and endoreduplication cycles of *Drosophila* embryogenesis. Genes Dev. *9*, 1327–1339.

Sigrist, S.J., and Lehner, C.F. (1997). Drosophila fizzy-related down-regulates mitotic cyclins and is required for cell proliferation arrest and entry into endocycles. Cell *90*, 671–681.

Smith, A.V., and Orr-Weaver, T.L. (1991). The regulation of the cell cycle during *Drosophila* embryogenesis—the transition to polyteny. Development *112*, 997–1008.

Stocker, H., and Hafen, E. (2000). Genetic control of cell size. Curr. Opin. Genet. Dev. *10*, 529–535.

Su, T.T., and O'Farrell, P.H. (1998). Chromosome association of minichromosome maintenance proteins in Drosophila endoreplication cycles. J. Cell Biol. *140*, 451–460.

Tada, S., Li, A., Maiorano, D., Mechali, M., and Blow, J.J. (2001). Repression of origin assembly in metaphase depends on inhibition of RLF- B/Cdt1 by geminin. Nat. Cell Biol. *3*, 107–113.

Traas, J., Hülskamp, M., Gendreau, E., and Höfte, H. (1998). Endoreplication and development: rule without dividing? Curr. Opin. Plant Biol. *1*, 498–503.

Urata, Y., Parmelee, S.J., Agard, D.A., and Sedat, J.W. (1995). A three-dimensional structural dissection of Drosophila polytene chromosomes. J. Cell Biol. *131*, 279–295.

Varmuza, S., Prideaux, V., Kothary, R., and Rossant, J. (1988). Polytene chromosomes in mouse trophoblast giant cells. Development *102*, 127–134.

Wang, Z., Zhang, Y., Kamen, D., Lees, E., and Ravid, K. (1995). Cyclin D3 is essential for megakaryocytopoiesis. Blood *86*, 3783–3788.

Weiss, A., Herzig, A., Jacobs, H., and Lehner, C.F. (1998). Continuous Cyclin E expression inhibits progression through endoreduplication cycles in *Drosophila*. Curr. Biol. *8*, 239–242.

White, M.J.D. (1973). Animal Cytology and Evolution, Third Edition (Cambridge: Cambridge University Press).

Whittaker, A.J., Royzman, I., and Orr-Weaver, T.L. (2000). Drosophila double parked: a conserved, essential replication protein that colocalizes with the origin recognition complex and links DNA replication with mitosis and the down-regulation of S phase transcripts. Genes Dev. *14*, 1765–1776.

Wohlschlegel, J.A., Dwyer, B.T., Dhar, S.K., Cvetic, C., Walter, J.C.,

and Dutta, A. (2000). Inhibition of eukaryotic DNA replication by geminin binding to cdt1. Science *290*, 2309–2312.

Zhang, Y., Wang, Z., Liu, D.X., Pagano, M., and Ravid, K. (1998). Ubiquitin-dependent degradation of cyclin B is accelerated in polyploid megakaryocytes. J. Biol. Chem. *273*, 1387–1392.

Zhang, H., Stallock, J.P., Ng, J.C., Reinhard, C., and Neufeld, T.P. (2000). Regulation of cellular growth by the *Drosophila* target of rapamycin dTOR. Genes Dev. *14*, 2712–2724.

Zimmet, J., and Ravid, K. (2000). Polyploidy: occurrence in nature, mechanisms, and significance for the megakaryocyte-platelet system. Exp. Hematol. *28*, 3–16.

Zimmet, J.M., Ladd, D., Jackson, C.W., Stenberg, P.E., and Ravid, K. (1997). A role for cyclin D3 in the endomitotic cell cycle. Mol. Cell. Biol. *17*, 7248–7259.

Zybina, E.V., and Zybina, T.G. (1996). Polytene chromosomes in mammalian cells. Int. Rev. Cytol. *165*, 53–119.