

Endoreplication and polyploidy: insights into development and disease

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Summary

Polyploid cells have genomes that contain multiples of the typical diploid chromosome number and are found in many different organisms. Studies in a variety of animal and plant developmental systems have revealed evolutionarily conserved mechanisms that control the generation of polyploidy and have recently begun to provide clues to its physiological function. These studies demonstrate that cellular polyploidy plays important roles during normal development and also contributes to human disease, particularly cancer.

Key words: Cancer, Endocycle, Endomitosis, Endoreplication, Genome instability

Introduction

Polyploid cells, which contain multiples of the diploid genome equivalent, have been studied for many years, nearly as long as chromosomes themselves have been studied. Now, over a century after the discovery of polyploidy, we know much about the molecular mechanisms that generate cellular polyploidy, but comparably little regarding the physiological function of the polyploid state. This discrepancy exists despite the frequent occurrence of polyploid cells in most multicellular organisms, as well as in human cancers. An important aspect of deciphering the roles for polyploidy lies in understanding the regulation of endoreplication, a cell cycle variation that generates a polyploid genome by repeated rounds of DNA replication in the absence of cell division. Recent advances show that, although some differences exist in the diverse endoreplication programs found from protists to humans, core principles can be applied. New work in genetic model organisms has identified cases in which programmed endoreplication plays key roles in development. Furthermore, recent evidence indicates that endoreplication can confer genome instability, a major cancer-enabling property. In this Primer (see Box), we review such recent discoveries, focusing on work carried out in the past 3 years, and refer the reader to other comprehensive reviews on this topic (De Veylder et al., 2011; Lee et al., 2009). From these new insights emerge unifying themes regarding endoreplication that hold promise of elucidating the advantages, as well as the potential disadvantages, of polyploidy.

Endoreplication and development

Endoreplication is typically studied in the context of endopolyploidy, which refers to the presence of polyploid cells in an otherwise diploid organism. Diverse levels of polyploidy occur throughout nature (Table 1). Although polyploidy is well

appreciated in plants (De Veylder et al., 2011), many different animal tissues, including the skin, gut, placenta, liver, brain and blood have polyploid cells (Table 1) (Laird et al., 1980; Corash et al., 1989; Fox et al., 2010; Hedgecock and White, 1985; Melaragno et al., 1993; Sherman, 1972; Unhavaithaya and Orr-Weaver, 2012; Zanet et al., 2010). Interestingly, endoreplication is not limited to multi-cellular organisms, with examples described in ciliated protozoa (Yin et al., 2010) and even bacteria (Mendell et al., 2008).

Two primary forms of endoreplication have been described: endocycling and endomitosis (Fig. 1). Endocycles are composed of alternating periods of S phase, during which DNA replication occurs, and gap phase, when cells prepare for the next round of S phase. Endocycling cells do not display any features of mitosis. Cells undergoing endomitosis, by contrast, execute an abortive mitosis that does not result in cell division, followed by subsequent re-entering of S phase. Although some molecular regulators of each cycle type may differ slightly across species, the core regulatory principles are essentially the same. Moreover, given new data showing that both endocycles and endomitosis exist in flies and mammals (Ullah et al., 2009b; Unhavaithaya and Orr-Weaver, 2012), and that varying degrees of both endocycles and endomitosis can occur in the same tissue, the distinction between the two may be insignificant given that they lead to the same outcome (i.e. polyploidy). Because of this similarity, we will refer to all polyploidizing cell cycles as 'endoreplication', unless a specific context dictates otherwise.

Cell cycle regulation of endoreplication

With respect to the cell cycle machinery, one of the most interesting features of endoreplication is that the same regulatory proteins that drive a typical cell division cycle are used to drive endoreplication. Developmental signals exploit this inherent plasticity of the cell cycle to convert a typical cell division cycle into an endoreplication cycle. To understand this process, we first need to describe some of the fundamental principles of cell cycle control and DNA replication, and then describe the mechanisms by which cells apply these principles to generate an endoreplication cycle.

A general overview of cell cycle control

Progression through the canonical G1-S-G2-M cell division cycle is controlled by a family of serine/threonine protein kinases, called cyclin-dependent kinases (CDKs). At the appropriate times, CDKs phosphorylate specific substrates to bring about the two major

Development: the big picture

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Table 1. Examples of the evolutionary diversity of polyploidy

| Organism* | Tissue/cell type | Ploidy | Feature or potential role played by polyploidy |
|---------------------|--------------------------|-------------|------------------------------------------------|
| <i>Drosophila</i> | Brain/subperineural glia | Up to 22.2C | Blood-brain barrier integrity |
| <i>Drosophila</i> | Hindgut/rectal papillae | Up to 16C | Chromosome mis-segregation during mitosis |
| <i>Drosophila</i> | Ovary/follicle cell | Up to 32C | Eggshell gene amplification |
| <i>Drosophila</i> | Salivary gland | Up to 2048C | DNA damage resistance |
| <i>M. musculus</i> | Liver/hepatocytes | Up to 16C | Localized genome under-replication |
| <i>M. musculus</i> | Keratinocytes | Up to 12C | Chromosome mis-segregation during mitosis |
| <i>M. musculus</i> | Megakaryocytes | Up to 32C | |
| <i>M. musculus</i> | Giant trophoblast | Up to 850C | DNA damage resistance |
| <i>C. elegans</i> | Intestine | 32C | |
| <i>Arabidopsis</i> | Leaf pavement cell | Up to 16C | |
| <i>Arabidopsis</i> | Leaf trichome | Up to 64C | Excessive endoreplication after DNA damage |
| <i>Tetrahymena</i> | Macronucleus | ~45C | Gene elimination |
| <i>Epulopiscium</i> | Symbiotic bacteria | ~40,000C | |

*This list of polyploid cell types is not comprehensive; many other tissues in these organisms contain polyploid cells.

events of the cell division cycle: S phase and mitosis. These two events are coupled such that S phase cannot begin until mitosis is completed. This coupling is enforced by CDK activity, which alternates between relatively low levels (in G1) and high levels (in S-G2-M) during the cell division cycle (Morgan, 2007) (see Box 1). Consequently, two important events must occur in order to convert a mitotic cycle into an endoreplication cycle and achieve polyploidy: (1) cell division must be suppressed; and (2) CDK activity must continue to alternate between low and high levels in order for the genome to be reduplicated in the absence of cell division. As we discuss below, different cell types use different mechanisms to accomplish these tasks.

Endoreplication by programmed inhibition of mitosis and cell division

Although transcriptional downregulation of mitotic regulators is one known mechanism for promoting endoreplication (Maqbool et al., 2010; Pandit et al., 2012; Zielke et al., 2008), endoreplicating cells can inhibit mitosis in a number of ways. One of the evolutionarily oldest mechanisms of mitotic inhibition, being conserved between plants and animals, is downregulation of mitotic CDK activity by ubiquitin-mediated proteolysis. Entry into and progression through mitosis requires the function of CDKs that are activated by A- and B-type cyclins. Conversely, exit from mitosis and subsequent cytokinesis requires the inhibition of these CDKs, which is achieved via cyclin proteolysis. This proteolysis is triggered by an E3 ubiquitin ligase called the anaphase-promoting complex/cyclosome (APC/C). An APC/C substrate targeting subunit, called Fzr/Cdh1 (Rap – FlyBase), directs APC/C^{Fzr/Cdh1} activity towards A- and B-type cyclins, beginning in late mitosis and continuing through the subsequent G1 phase, thus helping ensure efficient and irreversible exit from mitosis (Morgan, 2007; Song and Rape, 2011). Some endoreplicating cells take advantage of this mitotic suppression mechanism to achieve polyploidy. For example, Fzr/Cdh1 expression in *Drosophila* and *Arabidopsis* is both necessary and sufficient for endoreplication (Larson-Rabin et al., 2009; Sigrist and Lehner, 1997). *Drosophila* APC/C^{Fzr/Cdh1} activity is continuously required during endocycles to ensure suppression of mitotic regulators (Narbonne-Reveau et al., 2008; Zielke et al., 2008), and genetic evidence in *Arabidopsis* indicates that destruction of A- and B-type cyclins mediated by the Fzr/Cdh1 homolog CCS52A1 is crucial for endoreplication (Boudolf et al., 2009; Kasili et al., 2010). Moreover, the failure to properly control the timing of inhibition of APC/C activity during *Arabidopsis*

development results in inappropriate endoreplication (Iwata et al., 2011).

CDKs can also be inhibited through direct binding to proteins called cyclin kinase inhibitors (CKIs), and some polyploid cell types use CKIs to inhibit mitosis and trigger endoreplication (Ullah et al., 2009a). This is particularly true for mammalian trophoblast giant cells (TGCs) of the placenta and megakaryocytes, which generate platelets for blood clotting. When trophoblast stem cells differentiate into endoreplicating TGCs, induction of the p57 CKI results in inhibition of Cdk1 (Ullah et al., 2008). The p21 CKI plays a similar role in megakaryocyte endoreplication (Chagraoui et al., 2011; Muñoz-Alonso et al., 2012), as does the *SIAMESE* gene, which encodes an *Arabidopsis* CKI required for endoreplication in leaf hairs known as trichomes (Churchman et al., 2006; Morohashi and Grotewold, 2009). CKIs thus play an important part in the differentiation and terminal polyploid phenotype of diverse cell types.

Blocking cytokinesis is another mechanism that can promote endoreplication and polyploidy. For example, horticulturists have long used microtubule poisons, such as colchicine, to inhibit cell division and stimulate polyploid derivatives of important crop species (Hancock, 2005). The small GTPase RhoA is a key regulator of cell division, and multiple mechanisms ensure that RhoA is activated at the correct time and place to initiate cytokinesis (Glotzer, 2005). Megakaryocyte endoreplication requires the downregulation of two guanine nucleotide exchange factors, GEF-H1 and ECT2, that activate RhoA during cytokinesis, and their expression is also sufficient to prevent it (Gao et al.,

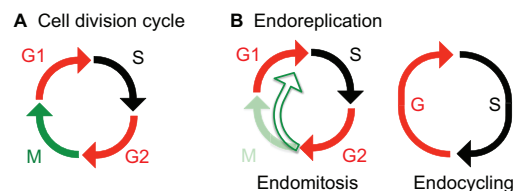


Fig. 1. Endoreplication results from cell cycle plasticity during development. (A) A canonical cell division cycle consists of four distinct phases. Chromosomes are duplicated during S phase and segregated to daughter cells during M phase. **(B)** Endoreplication can occur either by endomitosis, a cell cycle that displays features of mitosis but lacks cytokinesis, or by endocycling in which periods of S and G phase alternate with no cell division.

Box 1. CDK-mediated control of DNA replication

With respect to S phase, low CDK activity permits the assembly of replication initiation proteins into a 'pre-replicative complex' (pre-RC) on DNA at places where DNA replication will initiate once S phase begins. Pre-RC assembly during G1 allows DNA to become competent to replicate, which is referred to as 'replication licensing'. High CDK activity both initiates DNA replication during S phase and prevents DNA from becoming licensed to replicate during S and G2 phases by inhibiting assembly of the pre-RC (Diffley, 2011). Thus, only when CDK activity is abolished at the end of mitosis can the genome become licensed to re-enter S phase. This mechanism ensures that, during a normal cell division cycle, the genome is replicated once and only once – a requisite for maintaining diploidy.

2012). Endoreplication in rat liver also involves a developmentally regulated block to cytokinesis that occurs during weaning (Celton-Morizur and Desdouets, 2010; Celton-Morizur et al., 2010; Celton-Morizur et al., 2009).

An important concept emerging from these studies is that cells use several different mechanisms to suppress cell division and promote endoreplication. In addition, the combined use of multiple mechanisms to inhibit mitotic CDK activity and cytokinesis (e.g. transcriptional repression, ubiquitin-mediated proteolysis, CKI expression, RhoA inhibition) by a particular cell type ensures robust commitment to endoreplication.

The endoreplication oscillator: toggling between high and low CDK activity

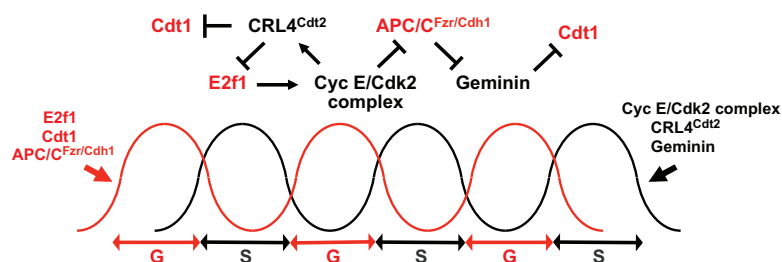
Replication of DNA during S phase requires CDK activity. Indeed, CKI induction during megakaryocyte differentiation must be transient in order not to inhibit the CDK activity required for endoreplication S phase (Muñoz-Alonso et al., 2012). Cdk2 is the important kinase for endoreplication S phase in animal cells, although in the absence of Cdk2 in mammals, Cdk1 can act as a substitute (Ullah et al., 2009b). Cyclin E overexpression increases the ploidy of megakaryocytes (Eliades et al., 2010), suggesting that the Cyclin E/Cdk2 (Cdc2c – FlyBase) complex is the relevant kinase. Likewise, early work suggested that the Cyclin E/Cdk2 complex is the crucial, and perhaps only, CDK required for endoreplication in *Drosophila* (Lilly and Duronio, 2005). However, Sallé et al. recently showed that Cyclin A regulates endoreplication S phase dynamics in *Drosophila* mechanosensory organs, although a corresponding CDK was not identified (Sallé et al., 2012). In spite of such important complexities, informative models of endoreplication can be built by considering the S-phase CDK as a single activity.

The alternation of high and low levels of CDK activity that is needed for endoreplication can often be observed cytologically. For example, Cyclin E transcripts and protein are present just prior to and during S phase, but not G phase, in endoreplicating *Drosophila*

cells (Knoblich et al., 1994; Lilly and Spradling, 1996; Weng et al., 2003). Endoreplication is suppressed when such cyclic accumulation of Cyclin E is bypassed by forced continuous transcription of *Cyclin E* (Follette et al., 1998; Weiss et al., 1998). The Cyclin E/Cdk2 complex also functions to control the cyclic accumulation of replication factors like the pre-RC component Orc1 (Narbonne-Reveau et al., 2008). These observations helped formulate the idea of an endoreplication oscillator that controls periods of high and low S-phase CDK activity.

Recently, mathematical modeling of endoreplication oscillations helped guide experiments demonstrating that cyclic accumulation of the transcription factor E2f1 (E2f – FlyBase) is essential for endoreplication in the highly polyploid *Drosophila* salivary gland (Zielke et al., 2011). E2F transcription factors are potent stimulators of S-phase entry and control the expression of genes required for DNA synthesis, including *Cyclin E*. Their activity is regulated by the Rb family of tumor suppressors, which bind to and inhibit E2F proteins during G1 phase (van den Heuvel and Dyson, 2008). E2F-Rb interactions regulate endoreplication in plants and worms (Magyar et al., 2012; Ouellet and Roy, 2007). However, Rb-mediated regulation of E2f1 is not essential for endoreplication in *Drosophila* salivary glands, perhaps because of the action of the E2f2-containing Myb-MuvB complex in repressing Cyclin E expression during G phase (Maqbool et al., 2010; Weng et al., 2003). Zielke et al. (Zielke et al., 2011) provide data in support of a model whereby E2f1 accumulation during G phase results in activation of the Cyclin E/Cdk2 complex, which triggers S phase, which in turn causes the subsequent inactivation of E2f1 via the action of CRL4^{Cdt2}, an E3 ubiquitin ligase that couples proteolysis with DNA replication (Havens and Walter, 2011; Shibutani et al., 2008) (Fig. 2). This model is consistent with earlier observations that Cyclin E activity is required for the downregulation of E2f1 target genes in the endoreplicating embryonic midgut (Duronio and O'Farrell, 1995), and that disruptions to normal oscillations of E2f1 activity in salivary glands suppress endoreplication (Maqbool et al., 2010). Interestingly, CRL4 is also required for endoreplication in *Arabidopsis* trichomes, although the relevant CRL4 target in trichomes might be CKI proteins (Roodbarkelari et al., 2010). Negative-feedback regulation of E2F activity is also important for endoreplication in hepatocytes (Chen et al., 2012; Pandit et al., 2012), but here it appears to control the transition from cell division to endoreplication, and it is not yet known whether E2F functions as part of a mammalian endoreplication oscillator. Nonetheless, these results emphasize that negative-feedback regulation is a common and important feature of molecular oscillators that control cell cycle progression (Ferrell et al., 2011).

A central CDK oscillator provides a mechanism through which other important aspects of endoreplication can be controlled (Fig. 2). For example, the oscillation of APC/C activity in *Drosophila* salivary glands probably results directly from the oscillation of Cyclin E/Cdk2 complex activity, which inhibits the

**Fig. 2. The endoreplication oscillator in *Drosophila* salivary glands.**

The core regulatory relationships between E2f1, Cyclin E and CRL4^{Cdt2} (top) give rise to out-of-phase oscillations in the activity of key regulatory proteins controlling S phase (bottom). E2f1, APC^{Fzr/Cdh1} and Cdt1 (red) are active during G phase, whereas the Cyclin E/Cdk2 complex, CRL4^{Cdt2} and Geminin (black) are active during S phase. Note that CRL4^{Cdt2} activity is dependent on DNA replication, which is triggered by the Cyclin E/Cdk2 complex, and that Cdt1 is inhibited both by Geminin binding and CRL4^{Cdt2}-mediated destruction.

Box 2. Genome instability

Genome instability refers to an aberrant state of frequent genome alteration. Diverse mechanisms contribute to genome instability. These include chromosome instability, a frequent mis-segregation of chromosomes during cell division (Negrini et al., 2010) or failed or improper repair of DNA damage (Lord and Ashworth, 2012). Because the genome alterations associated with such instability increase the frequency of gene mutation or enable gene overexpression, genome instability is considered the most significant enabling feature of cancer (Hanahan and Weinberg, 2011).

APC/C (Narbonne-Reveau et al., 2008). Key targets of the APC/C during endoreplication progression are the mitotic cyclins and the protein Geminin, which is an inhibitor of the essential replication licensing protein Cdt1 (Dup – FlyBase). Indeed, the combination of mitotic Cdk1 inhibition and Geminin proteolysis can trigger endoreplication in human cells (Hochegger et al., 2007). By contrast, in *Arabidopsis*, little or no APC/C activity is required for endoreplication progression (as opposed to endoreplication entry, as described above), perhaps because there is no Geminin protein (Roodbarkelari et al., 2010). In *Drosophila* salivary glands, Geminin is absent during G phase, when replication licensing occurs, and present during S phase to prevent DNA licensing (Zielke et al., 2008). In this way, the endoreplication S phase is similar to the S phase of a cell division cycle and distinct from the phenomenon of re-replication, in which specific segments of DNA are replicated more than once in a single S phase. In contrast to endoreplication, re-replication is an aberrant situation that causes genomic instability and is observed in some cancer cells (see Box 2).

In summary, the central endoreplication oscillator entrains many molecular activities to ensure the cycling of CDK activity and, thus, the cycling of replication licensing. It is important to keep in mind that the mechanism by which cyclic CDK activity is achieved may differ among species or even among different cell types within the same organism. For example, *Drosophila* nurse cells probably rely more on CKI activity than on E2F activity for CDK control during endoreplication (Hong et al., 2007). Nevertheless, the network of interactions controlling cyclic S phase CDK activity constitutes the central principle of endoreplication that we propose is universally applicable.

Functions of endoreplication and polyploidy

Given the prevalence of polyploid cell types in many different organisms, a reasonable expectation is that endoreplication provides key biological functions during development. Surprisingly, there are relatively few examples of a biological function that is specifically attributed to endopolyploidy. A reason may be that the ideal experiment – converting a tissue composed of polyploid cells into a tissue of the same size composed of diploid cells – is not experimentally trivial and requires a full understanding of endoreplication mechanisms. However, several recent exciting studies have begun to provide evidence that polyploidy is an important functional adaptation in a variety of tissues.

Regulation of the mitosis-to-endoreplication switch

To understand the purpose of endoreplication, we must understand how and when endoreplication initiates. Furthermore, by inhibiting the switch from mitosis to endoreplication, we can learn about the purpose of polyploidy. Perhaps not surprisingly, signal transduction

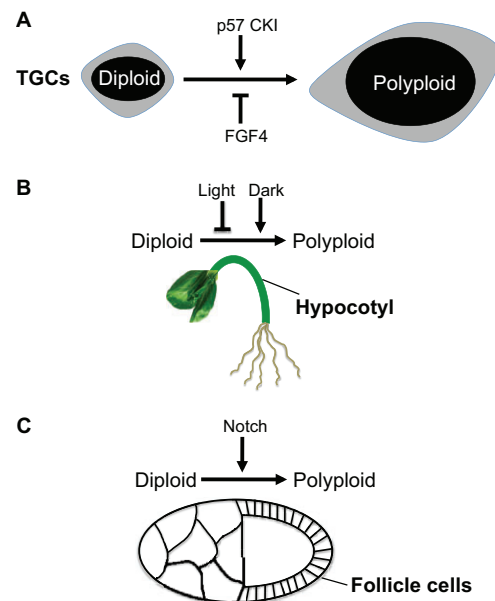


Fig. 3. Signals triggering polyploidy. (A–C) Signals that regulate the mitosis-to-endoreplication switch in mammals (A; trophoblast giant cells, TGCs), plant seedlings (B; hypocotyl) and insects (C; *Drosophila* ovarian follicle cells).

pathways control this switch, and these signals are as variable as the cell types that use them (Fig. 3). These signals control both the decision to make the switch, and the activity of cell cycle regulators that will execute the switch. For example, some of the earliest observations were made in *Drosophila* follicle cells, which respond to Notch signaling to activate *Fzr/Cdh1* expression and suppress Cdk1 activity (Deng et al., 2001; Shcherbata et al., 2004; Sun and Deng, 2007). Recent work in this *Drosophila* model tissue suggests that chromatin modifications may accompany the Notch-regulated mitosis-to-endoreplication switch (Domanitskaya and Schüpbach, 2012). The involvement of Notch in regulating endoreplication appears conserved, as mammalian megakaryocyte differentiation is regulated in part by Notch, although the nature of this regulation (positive versus negative) may differ between humans and mice (Cornejo et al., 2011; Mercher et al., 2008; Poirault-Chassac et al., 2010).

In addition to Notch, recent advances in *Arabidopsis* and mammalian cell culture systems have identified additional signals that promote the mitosis-to-endoreplication switch. An important principle emerging from these studies is that the developmental signals controlling endoreplication are the very same signals that control other aspects of the cell biology of specific cell types; in other words, endoreplication is just one aspect of the phenotype of differentiated cells. For example, the depletion of fibroblast growth factor 4 (FGF4) from trophoblast stem cells triggers both TGC development and endoreplication (Ullah et al., 2008), as does insulin signaling in hepatocytes (Celton-Morizur et al., 2009).

Plant cells trigger endoreplication using developmental signals that are not relevant to animals, two of the most important of which are light and the plant hormone auxin. Light promotes cell division in hypocotyls, and activates the transcription of *DEL1*, which encodes an atypical E2F that inhibits endoreplication by repressing the expression of *CCA52A1* (*Fzr/Cdh1*). In the dark, *DEL1* expression is extinguished and hypocotyl cells endoreplicate

(Berckmans et al., 2011). In the root meristem (which is not exposed to light), auxin signaling regulates the mitosis-to-endoreplication switch (Ishida et al., 2010). Correct timing of this switch is also important, as precocious endoreplication can have drastic consequences. Mutation of the SUMO E3 ligase HPY2 results in stunted root growth because of an increase in cellular ploidy at the expense of cell proliferation (Ishida et al., 2009). Conversely, a failure to switch to endoreplication inhibits trichome cell fate commitment and disrupts normal leaf development (Bramsiepe et al., 2010). A similar connection between cell fate and endoreplication has also been observed in the *Drosophila* germ line (Lilly and Spradling, 1996).

Consequences of defective endoreplication during development

Polyploid cells are typically large, and endoreplication can thus be considered as a form of tissue growth. Consequently, the pathways regulating cellular growth play important roles in the control of endoreplication (Demontis and Perrimon, 2009; Pierce et al., 2008; Steiger et al., 2008). But what might be the advantages of cell growth mediated via polyploidy? One idea is that more copies of the genome increase the biosynthetic capability of cells. A good example is provided by polyploid *Drosophila* follicle cells, which require many copies of the chorion genes to produce sufficient protein for eggshell biosynthesis (Calvi and Spradling, 1999). However, increased gene copy number does not always correlate with increased gene expression (Kim et al., 2011). Thus, the idea that polyploidy primarily increases biosynthetic capacity is probably too simplistic.

In addition to increasing gene copy number, endoreplication can alter genome structure in unexpected ways. In *Drosophila*, many endoreplicating cells fail to complete late S phase, leaving some genome regions dramatically under-replicated, including heterochromatic sequences (Gall et al., 1971; Lilly and Spradling, 1996). Using genomic deep-sequencing and chromatin immunoprecipitation approaches, recent studies of polyploid *Drosophila* genomes precisely defined a number of under-replicated regions also present in euchromatin (Nordman et al., 2011). Most of these under-replicated regions correlate with marks of transcriptional repression (Sher et al., 2012). Interestingly, the degree and location of endoreplication-induced under-replication is dependent on tissue type, suggesting a role for under-replication in defining tissue identity (Nordman et al., 2011). However, under-replication can be blocked by mutation in a chromatin protein Suppressor of Under-Replication (SuUR) and *SuUR* mutant flies exhibit no obvious alteration of polyploid tissue function (Belyaeva et al., 1998). Thus, unlike gene amplification, the role of under-replication in polyploid tissue function remains unclear. It remains possible that under conditions of physiological stress, a role for this common yet poorly understood polyploid genome alteration may be uncovered. Nevertheless, coupled with studies of programmed DNA elimination in the polyploid macronucleus of ciliates (Chalker and Yao, 2011), under-replication and amplification all represent examples of ‘genome flexibility’ exhibited by polyploid cells. Further study of such flexibility may lead to novel functions of endoreplication in development.

There are aspects of growth that might be best served by endoreplication rather than cell proliferation. A beautiful example is provided by a recent study of the *Drosophila* larval nervous system. Here, growth via endoreplication is required for glial cells to maintain tight junctions, and thus the blood-brain barrier, during proliferative expansion of the underlying brain tissue

(Unhavaithaya and Orr-Weaver, 2012). Interestingly, these glial cells become polyploid either via endocycles or endomitosis, suggesting that the important outcome is growth via endoreplication rather than cell proliferation, regardless of the cell cycle mechanism. Other cell types regulate endoreplication to control organ shape in addition to growth. This is particularly true for the branched trichome structures of *Arabidopsis* leaves (Kasili et al., 2011), which can also balance proliferation with endoreplication to regulate size and shape (Li et al., 2009). The emerging theme is that the correct timing and extent of endoreplication is often an essential aspect of tissue growth and morphogenesis in both animals and plants.

In spite of these exciting examples, the function of endoreplication is not always forthcoming. Mammalian livers have a remarkable capacity for regeneration after partial hepatectomy, and regeneration can occur via Cyclin E-mediated regulation of endoreplication rather than proliferation (Miyaoka et al., 2012; Nevzorova et al., 2009). Because many hepatocytes are normally polyploid, a reasonable hypothesis is that endoreplication is important for normal liver function. However, recent studies in mouse show that shifting the hepatocyte population from polyploid cells to diploid cells by manipulating E2F activity does not dramatically affect liver function or regeneration (Chen et al., 2012; Pandit et al., 2012). This was somewhat surprising, and suggests that our understanding of the relationship between polyploidy and normal biological function requires further investigation. The following sections describe emerging evidence suggesting an intriguing and complex relationship between polyploidy, genome stability and cancer.

Endoreplication and disease

Propagating a stable genome is part of normal cell cycle progression and is required for successful development. It follows that non-canonical cell cycle progression and genome instability (see Box 2) are linked phenomena. Interestingly, recent work from numerous systems suggests a reciprocal connection between genome instability and endoreplication: genome instability promotes endoreplication and, vice versa, endoreplication promotes genome instability (Fig. 4). These findings implicate endoreplication as both an adaptation to genotoxic stress, as well as a potentially novel mechanism for differentiation of polyploid cells. Furthermore, the parallels between normal and cancerous

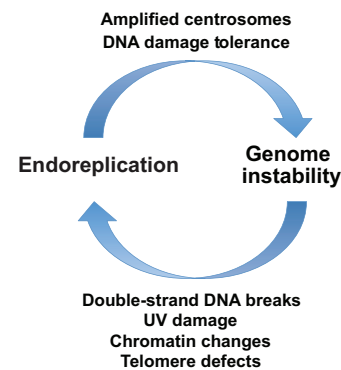


Fig. 4. The reciprocal relationship between endoreplication and genome stability. Endoreplication can promote genome instability in the form of amplified centrosomes and an increased tolerance for DNA damage. Conversely, DNA damage, chromatin changes and telomere defects are all examples of genome instability forms that have been demonstrated to initiate endoreplication.

endoreplication cycles highlight that the study of developmentally programmed endoreplication may illuminate mechanisms by which polyploidy promotes precancerous genome alteration.

Genome instability promotes endoreplication

A damaged genome presents a challenge for a developing or regenerating tissue. During these dynamic events, tissue mass must be increased, but cell proliferation must be delayed to avoid segregation of damaged chromosomes. Emerging data from multiple systems now suggest a conserved role for endoreplication in providing an alternative to deleterious proliferation in the face of genome instability.

Recent evidence suggests that plants possess endoreplication-promoting mechanisms that enable tissue growth despite genome damage. Following induction of double-stranded DNA breaks in *Arabidopsis* root tip and sepal cells, normally mitotic cells increase in size via endoreplication. This cell cycle alteration requires the plant-specific transcription factor SUPPRESSOR OF GAMMA RESPONSE 1 (SOG1), which transmits signals from the conserved ATAXIA TELANGIECTASIA MUTATED (ATM) and ATAXIA TELANGIECTASIA-MUTATED AND RAD3-RELATED (ATR) DNA damage sensor kinases. This DNA break response elicits transcriptional changes that are consistent with downregulation of mitotic factors and upregulation of cell cycle genes that can promote endoreplication (Adachi et al., 2011). Similarly, *Arabidopsis* leaves possess an endoreplication response to genome damage (Radziejowski et al., 2011). Following UV irradiation, mRNA levels of E2Fe/DEL1, an atypical E2F protein, decrease. Mutant studies showed that loss of E2Fe/DEL1 elevates transcription of PHOTOLYASE 1 (PHR1), a regulator of UV-induced DNA repair. Plants that lack E2Fe/DEL1 exhibit enhanced repair of UV-induced breaks and also increased endoreplication (Radziejowski et al., 2011). Taken together, these recent studies argue that endoreplication is a favorable mode of plant tissue growth following genotoxic stress.

In both plants and animals, genome instability in the form of defective chromatin assembly is also tied to endoreplication. In *Arabidopsis*, mutations in *FASCIATA1* (*FAS1*), which encodes a subunit of the CHROMATIN ASSEMBLY FACTOR 1 (CAF1) histone chaperone, triggers an inappropriate mitosis to endoreplication switch. These mutations specifically block binding of FAS1 to E2F family members. As a result of this blocked interaction, genes known to regulate the S/G2 checkpoint are upregulated, which is likely to promote endoreplication (Ramirez-Parra and Gutierrez, 2007). Similarly, depletion of *Drosophila* CAF-1 leads to death in diploid cells but does not block endocycle progression (Klapholz et al., 2009). In both flies and plants, CAF1 loss correlates with DNA damage, providing further evidence of endoreplication as an adaptation to genome damage.

Telomere shortening, which also activates the DNA damage response, appears to be a potent endoreplication-promoting mechanism in many normally proliferative cells. Using an inducible system that activates a persistent DNA damage response at telomeres, Davoli et al. (Davoli et al., 2010) showed that mouse embryonic fibroblasts increase in ploidy via endoreplication. This endoreplication requires ATM/ATR and occurs in the absence of the transcription factor p53, a known suppressor of S phase following polyploidy (reviewed by Aylon and Oren, 2011). These endoreplication cycles resemble developmentally programmed endocycles, exhibiting cyclic Geminin expression and a lack of mitotic CDK activation (Davoli et al., 2010). Inefficient telomere replication can also cause endoreplication. In human mammary

epithelial cells, shortened telomeres cause endoreplication through anaphase bridge formation and subsequent cytokinesis failure (Pampalona et al., 2012). Similar to the Davoli et al. study (Davoli et al., 2010), the endoreplication observed in the study by Pampalona et al. (Pampalona et al., 2012) requires aberrant (short) telomeres, but also requires loss of the transcriptional repressor Rb, rather than loss of p53. Telomere replication defects also contribute to endoreplication through failed chromosome segregation in mouse embryonic fibroblasts lacking the chromosome cohesion component SA1 (Remeseiro et al., 2012). These telomere studies provide potential mechanistic insight into how some animal tissues respond to DNA damage. Intriguingly, mice with damaged telomeric DNA regenerate their liver via endoreplication (Lazzerini Denchi et al., 2006). Given the connection between telomere shortening and aging, it is interesting to speculate that endoreplication may be a favored mode of tissue repair in aged humans.

How do cells employ endoreplication to continue the cell cycle despite DNA damage? Regulation of the transcriptional repressor Rb may provide a key mechanistic link. Loss of Rb contributes to tetraploidization following telomere crisis in human fibroblasts and mammary epithelial cells (Davoli and de Lange, 2012). During liver regeneration in normal mice, cell cycling is blocked in hepatocytes with damaged genomes. By contrast, Rb mutant hepatocytes with DNA damage continue to cycle and frequently endoreplicate, as evidenced by the emergence of polyploid cells. Interestingly, this endoreplication phenotype appears specific to Rb loss in the liver and not in other gastrointestinal tissues, suggesting that tissues exhibit differing capacities and requirements for endoreplication (Bourgo et al., 2011). Future work is needed to unravel how diverse genotoxic stresses combine with tissue-specific cell cycle regulation to promote endoreplication.

Endoreplication promotes genome instability

In normal polyploid tissues, programmed endoreplication is usually a permanent departure from a mitotic program. As a result, endoreplication has become synonymous with terminal differentiation. Conversely, polyploidy is associated with mitotic progression of many cancers (Davoli and de Lange, 2011). Experimentally induced endoreplication can enable chromosome instability (CIN), whereby chromosomes mis-segregate during mitosis, and such polyploid instability is associated with tumor growth in mice (reviewed by Ganem and Pellman, 2007). Thus, a simplistic view is that normal endoreplication promotes senescence and differentiation, whereas unscheduled endoreplication promotes tumorigenesis by enabling CIN. In contrast to this view, recent work in both insects and mammals has now shown that polyploid cells can resume mitotic cycles. Surprisingly, these polyploid mitotic cells exhibit CIN, similar to cancerous polyploid cells. This new finding suggests parallels between normal and cancerous endoreplication, but in addition provokes new ideas about apparently 'unstable' mitotic divisions contributing to the physiology of normal tissues containing polyploid cells.

During *Drosophila* larval development, endoreplication occurs frequently in many tissues. The resulting polyploid cells function in the larva but do not proliferate and contribute to the adult fly, with some recently studied exceptions. Fox et al., for example, discovered that intestinal papillar cells, which reclaim up to 90% of ingested water in an insect, form by proliferation of polyploid precursors, which had previously undergone endoreplication (Fox et al., 2010). Thus, the transition from mitotic cycles to endoreplication appears to be reversible in normal animal

development. Unexpectedly, these developmentally programmed divisions exhibit frequent signs of CIN, including chromosomal aberrations, anaphase bridging and lagging chromosomes. A similar propensity for CIN was observed in the hind-intestine of mosquitoes (Fox et al., 2010), as well as during endoreplication in *Drosophila* blood-brain barrier glial cells, which exhibit anaphase bridging during endo-mitotic cycles (Unhavaithaya and Orr-Weaver, 2012). The developmentally tractable nature of these CIN-prone cell cycles provides an accessible entry point into the study of mechanistic connections between endoreplication and genome instability.

The propensity for mitotic instability following endoreplication appears conserved. The liver has long been observed to contain endoreplicating cells, and recent evidence shows that these cells also revert to mitotic cycles that become error prone. Using FACS purification and a marked cell transplantation model, Duncan et al. followed polyploid mouse hepatocytes over time (Duncan et al., 2010). These transplanted hepatocytes frequently produced aneuploid cells (cells with an imbalance in chromosome content). This aneuploidy also occurs in normal mouse and human livers, as verified by karyotyping and *in situ* hybridization (Duncan et al., 2012a; Duncan et al., 2010). The intriguing observation of polyploid-induced aneuploidy in healthy tissues led the authors of these studies to propose that the transition from endoreplication to polyploidy and finally to aneuploidy represents a means of generating a genetically diverse pool of cells, a subset of which might be amplified under conditions of stress (Duncan et al., 2010). A recent follow up study presented one case in which this model holds true. In a mouse model liver disease model (tyrosinemia-induced injury), cells with a specific aneuploidy containing an extra disease-resistance gene copy can be amplified to suppress liver injury (Duncan et al., 2012b). These recent liver studies present a highly novel role for endoreplication in cellular differentiation.

How does endoreplication drive genome instability? One source is the duplication of the mitotic spindle organizers – the centrosomes. Because centrosome duplication is tied to cell cycle progression, passage through endoreplication could generate multiple centrosomes and aberrant mitotic spindles – a long-hypothesized source of genome instability (Holland and Cleveland, 2009). This appears to be the case in mouse hepatocytes: polyploid hepatocytes with extra centrosomes exhibit cell division errors, owing to apparently improper microtubule attachments (Duncan et al., 2010).

Recent studies in non-mitotic polyploid cells of *Drosophila* and mice suggest that endoreplication enables tolerance of DNA damage. In flies, endoreplicating cells acquire resistance to DNA damage through a mechanism involving the silencing of cell death genes (Mehrotra et al., 2008). Similarly, endoreplicating mouse trophoblast cells also downregulate the DNA damage response. During the differentiation of trophoblast stem cells into polyploid TGCs, protein levels of the damage-responsive kinase Chk1 diminish, permitting endoreplication. This decrease in Chk1 enables polyploid trophoblast cells to evade apoptosis through suppression of the DNA damage pathway (Ullah et al., 2011; Ullah et al., 2008). Taken together, it is clear that cell cycle alterations tied to endoreplication can encourage genome instability. The consequences of such polyploid genome instability on normal tissue development, as well as in diseases such as cancer, remain to be fully understood.

Endoreplication in cancer

As mentioned above, endoreplication can confer potentially precancerous genome instability but can also represent a permanent

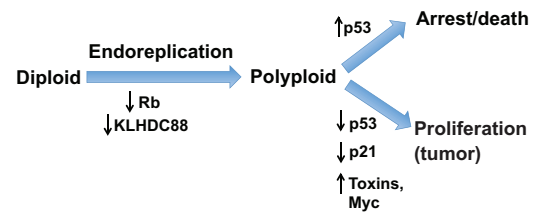


Fig. 5. Potential molecular mechanisms of polyploid tumor formation.

Distinct molecular alterations can influence each step of the progression from diploid to polyploid to tumorous proliferation. Shown here are recently identified mechanisms implicated in this progression. Genetic factors such as Rb loss or KLHDC88 mutation can promote endoreplication. Once cells achieve polyploidy, p53 can mediate cell cycle arrest or cell death. Conversely, in the absence of p53 or its target p21, or following administration of toxins such as diethylnitrosamine or aflatoxin B1 (see Reed et al., 2009 and McClendon et al., 2011), or in the presence of increased Myc (see Saddic et al., 2011), tumorigenesis may result.

block to mitosis. Following endoreplication, a polyploid cell may thus either arrest the cell cycle or regain potentially cancerous proliferation. Recent evidence from diverse models and humans with cancer now suggests that regulation of this decision plays a role in cancer progression.

Whether endoreplication suppresses or enables tumorigenesis appears to be dependent on the genetic background and tissue environment (Fig. 5). In mice, Cdk1 deletion results in liver regeneration solely through endoreplication, and correlates with decreased tumor risk (Diril et al., 2012). Similarly, loss of Rb (Rb1 – Mouse Genome Informatics) during mouse liver development promotes increased ploidy but does not promote spontaneous liver cancer, even in combination with p53 (Trp53 – Mouse Genome Informatics) loss (McClendon et al., 2011). However, this *p53*, *Rb* double mutant background renders mice highly susceptible to carcinogen-induced liver cancer (McClendon et al., 2011). Furthermore, both ploidy and liver tumorigenesis increase in Rb mutant mice in conjunction with either overexpression of the Myc transcription factor (Saddic et al., 2011) or a liver toxin (Reed et al., 2009). Outside the liver, inappropriate endoreplication may be more tumorigenic. Mouse embryonic cells lacking *Rb* and *p53* undergo endoreplication with de-protected telomeres, and subsequent division of such cells promotes aneuploidy and tumorigenesis (Davoli and de Lange, 2012). Future studies will clarify how genetic background and tissue environment influence whether endoreplication promotes or suppresses tumorigenesis.

In parallel to the above mouse studies, recent work has found that, in certain contexts, cancerous cells use endoreplication as a path to drug resistance. Several cancer cell lines treated with growth suppressing drugs enter endoreplication cycles (Sakaue-Sawano et al., 2011; Shen et al., 2008). This switch to endoreplication can be reversed, allowing the now polyploid cancer cells to resume proliferation and acquire resistance to apoptosis-inducing drugs (Shen et al., 2008). This endoreplication and subsequent proliferation requires inhibition of the p53 target p21 (Shen and Maki, 2010; Zheng et al., 2012). Thus, endoreplication may confer additional instability to acquire cancerous proliferative potential.

Many human cancers exhibit polyploidy (reviewed by Davoli and de Lange, 2011; Storchova and Pellman, 2004), but finding direct clinical links to endoreplication is challenging. Recent

analysis of multiple related individuals with Hodgkin's lymphoma led to the identification of a mutation in the cytokinesis regulator KLHDC8B (Salipante et al., 2009). In such individuals, a signature of disease progression is the accumulation of polyploid binucleate Reed-Sternberg cells. Depletion of KLHDC8B generates binucleate cells through endoreplication (failed cytokinesis). This study provides a genetic link between inappropriate, transient endoreplication and cancer progression. Future efforts in personal cancer genome sequencing may be key to identifying additional mutations that enable cancer through endoreplication.

Conclusions

The exciting recent progress highlighted here has re-invigorated efforts to understand the biological functions of endoreplication and polyploidy. Thanks to the development of new tissue models, as well as additional mechanistic insights in familiar systems, it now appears clear that unifying principles apply to all forms of endoreplication, be they 'endocycles', 'endomitosis' or 'failed mitosis'. We propose that future efforts should focus on an improved understanding of how tissues benefit from increasing the ploidy of a single cell rather than generating more cells via proliferation. From the recent work highlighted here, we now know that endoreplication can be advantageous to maintain both tissue and genome integrity in cases where proliferation would be disruptive. Furthermore, the continued study of mechanisms connecting endoreplication and genome instability may prove useful in understanding the mysterious role of polyploidy in cancer.

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Competing interests statement

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