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**Review**

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# Endomitosis: a new cell fate in the cell cycle leading to polyploidy in megakaryocytes and hepatocytes

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**Abstract:** Megakaryocytes and hepatocytes are unique cells in mammals that undergo polyploidization through endomitosis in terminal differentiation. Many polyploidization regulators and underlying mechanisms have been reported, most of which are tightly coupled with development, organogenesis, and cell differentiation. However, the nature of endomitosis, which involves successful entry into and exit from mitosis without complete cytokinesis, has not been fully elucidated yet. We highlight that endomitosis is a new cell fate in the cell cycle, and tetraploidy is a critical stage at the bifurcation of cell fate decision. This review will summarize the recent research progress in this area and provide novel insights into how cells manipulate mitosis toward endomitosis. Endomitotic cells can evade the tetraploidy restrictions and proceed to multiple rounds of the cell cycle. This knowledge not only deepens our understanding of endomitosis as a fundamental biological process but also offers new perspectives on the physiological and pathophysiological implications of polyploidization.

**Key words:** Polyploidy; Endomitosis; Megakaryocyte; Hepatocyte; Cell Cycle; Tetraploidy Checkpoint; Whole-genome Doubling

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## 1 Introduction

Whole-organism polyploidy is commonly found in plants, fungi, and lower animals, where it is linked to diversity and evolution (Albertin and Marullo, 2012; Van de Peer et al., 2017). In contrast, whole-organism polyploidy in mammals is not common and is highly debated, as it typically leads to embryonic resorption or spontaneous abortion (Svartman et al., 2005). At the cellular level, polyploidy is a distinct feature of specifically and highly differentiated mammalian cell types, such as skeletal muscle cells (Abmayr and Pavlath, 2012), osteoclasts (Loutit and Nisbet, 1982), trophoblasts (Pandit et al., 2013), megakaryocytes (Ravid et al., 2002; Mazzi et al., 2018), hepatocytes (Guidotti et al., 2003; Wang et al., 2017; Donne et al., 2020; Sladky et al., 2021), and cardiomyocytes (Gan et al., 2020). Polyploidy is tightly relevant to the physiological functions of these cells (Pandit, et al., 2013). For instance, the degree of polyploidy is associated with the capability of megakaryocytes to produce platelets (Zimmet and Ravid, 2000; Bluteau et al., 2009). Polyploidy in the liver is believed to be associated with increased metabolic capacity (Fox and Duronio, 2013; Van de Peer, et al., 2017). Meanwhile, the impact of polyploidy on hepatocellular carcinoma remains controversial (Zhang et al., 2018b; Lin et al., 2020; Sladky et al., 2020b; Lin et al., 2021), which is attributed to the different genetic landscapes, such as p53 mutation status (Bou-Nader et al., 2020; Sladky, et al., 2020b). Osteoclasts depend on polyploidy for their

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effector function in bone resorption (Loutit and Nisbet, 1982). In the lactating mammary gland, binucleated polyploid epithelial cells are required for efficient milk production (Rios et al., 2016). Precise regulation of polyploidization in these cells is critical for health, and dysregulation of polyploidization involves many diseases including cancer.

## 2 Route to polyploidy in mammalian cells

Polyploidy can be achieved through cell fusion, which is not dependent on the cell cycle and cell division. Endoreplication is another way to produce polyploidy, which continuously multiplies the genome without cell division. Endocycle (cycling cells proliferate without M phase) and endomitosis (cycling cells exit M phase and reenter G1 phase without cell division) are two major forms of endoreplication. Trophoblast giant cell polyploidization is a typical example of endocycle (Zielke et al., 2013). Endomitosis is mainly found in megakaryocytes and hepatocytes (Nevzorova et al., 2009; Mazzi, et al., 2018; Donne, et al., 2020; Vainchenker and Raslova, 2020; Sladky, et al., 2021; Sanz-Gómez et al., 2023). Notably, cytokinesis failure is sometimes regarded as a subtype of endomitosis (Zielke, et al., 2013). Therefore, we will use "endomitosis" as a collective term to refer to a diversity of cell cycle programs in which cells progress to and exit M phase without complete cytokinesis (Herriage et al., 2024). In this review, we choose megakaryocytes and hepatocytes as two representative cell types that achieve their polyploidization via endomitosis. The canonical mechanisms have been well reviewed in other articles (Suraneni and Crispino, 2016; Mazzi, et al., 2018; Donne, et al., 2020; Vainchenker and Raslova, 2020; Sladky, et al., 2021), which will be concisely summarized in this review. We will further highlight endomitosis as a new cell fate in the cell cycle.

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## 3 Canonical mechanisms of polyploidization

### 3.1 Megakaryocytes

Megakaryocytes are large (50–100 μm in diameter), rare (0.05%–0.1%), polyploid, and platelet-producing hematopoietic cells that predominantly reside in the bone marrow in adults (Ebaugh and Bird, 1951). Recently, megakaryocytes have also been found in the lung and produce about 30% of platelets (Scheinin and Koivuniemi, 1963; Lefrancais et al., 2017). Megakaryocytes have a ploidy ranging from 2N to 128N, with a modal ploidy of 16N (Ravid, et al., 2002). Megakaryocytes are differentiated from hematopoietic stem cells (HSC), and megakaryocyte polyploidization is tightly coupled with differentiation and maturation, which are precisely regulated by many cytokines, signaling pathways, transcription factors, and cell cycle regulators.

#### 3.1.1 Cytokines and signaling pathways

Thrombopoietin (TPO) is the primary cytokine regulating the entire process of megakaryopoiesis and thrombopoiesis (Nagata et al., 1997). TPO primarily signals through the Janus kinase/signal transducer and activator of transcription (JAK/STAT), phosphoinositide 3-kinase (PI3K)/AKT, and mitogen-activated protein kinase (MAPK) pathways (Chanprasert et al., 2006). TPO signaling through STAT proteins regulates the expression of cyclins D and E along with BCL-XL (Eliades et al., 2010; Kaushansky, 2016) and induces modest increases in p21CIP1/WAF1, which may function as a licensing factor at low levels (Kikuchi et al., 1997). *Stat1*-deficient mice exhibit defects in ploidy, suggesting the importance of STAT1 in megakaryocyte polyploidization (Huang et al., 2007). STAT3 and STAT5 are two important downstream mediators of MPL/TPO signaling that promote megakaryocyte development and proliferation (Yu et al., 2016). Furthermore, TPO signals to PI3K to increase polyploidy by facilitating cell cycle progression through the PI3K/Akt/Foxo3a/p27Kip1 pathway (Nakao et al., 2008). PI3K, STATs, and MAPK pathways also regulate MYC expression (Chanprasert, et al., 2006). The role of mTOR (mTORC1) and MAPK signaling remains

controversial. One study demonstrated that mTOR inhibition had no effect on polyploidization (Drayer et al., 2006). However, another study demonstrated that mTOR induced enhanced polyploidization by inducing cyclin D3 and causing moderate upregulation of p21CIP1/WAF1 (Raslova et al., 2006). Studies have reported that MAPK/ERK (extracellular signal-regulated kinase) inhibition restricts ploidy and differentiation in human cell lines and mouse primary cells (Rouyez et al., 1997; Mazharian et al., 2009), but it does not show any effect or enhancement in human primary cells (Guerriero et al., 2006). Distinctions between human and murine megakaryocyte polyploidization responses to ERK signaling cannot be excluded. The negative effects of lysyl oxidase propeptide on polyploidization may be associated with the suppression of MAPK (Eliades et al., 2013). We and others have identified regulators in these signaling pathways, including ANP32A, PSTPIP2, and LNK, that affect normal and abnormal megakaryopoiesis (Tong and Lodish, 2004; Liu et al., 2014; Sun et al., 2017). Lastly, TPO may also modulate ploidy by inducing the formation of reactive oxygen species through the up-regulation of NADP oxidase NOX1 (Mccrann et al., 2009). Inhibition of various NOX isoforms decreases polyploidization and the expression of cyclins D3 and E (Mccrann, et al., 2009).

Many other hematopoietic cytokines also participate in megakaryocyte polyploidization. Members of the IL-6 (interleukin-6) family, including IL-6 and IL-11, promote polyploidy, whereas cytokines such as IL-3 can inhibit polyploidization. (Burstein et al., 1992). Although all of these signals converge on JAK2 or JAK1 to activate STAT proteins, differences in the activation of STAT isoforms, signal kinetics, or receptor expression levels may contribute to these varied effects. In vitro studies have shown that the inhibition of TGF-β (transforming growth factor beta-1) signaling enhances TPO-driven polyploidization (Kuter et al., 1992). This effect is possibly mediated through increased expression of CDKIs, such as p27KIP1, p57KIP2, or p15INK4B. The CXCL12/CXCR4 pathway may regulate polyploidization, but the role of CXCL12 in megakaryopoiesis remains contradictory (Hamada et al., 1998; Guerriero et al., 2001; Berthebaud et al., 2005; Stegner et al., 2017).

Diverse signaling pathways may also be involved in megakaryopoiesis and polyploidization, for instance, NF-κB (nuclear factor-kappa beta) and Notch signaling (Zhang et al., 2002; Sun et al., 2004; Mercher et al., 2008; Weiss-Gayet et al., 2016). PAK2, the CDC42/RAC pathway target, is suggested to restrain polyploidization in mice (Kosoff et al., 2015). Nicotinamide has been reported to elevate megakaryocyte ploidy in vitro, potentially through Sirtuin 1/2 inhibition (Giammona et al., 2009). The loss of MNK1 (MAPK-interacting kinase 1) in mice and in human cultured megakaryocytes reduces megakaryocyte ploidy in both models (Manne et al., 2022).

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### 3.1.2 Transcription factors

Megakaryocyte differentiation and polyploidization are tightly regulated by lineage-specific transcription factors, including GATA-1, TAL1 (SCL), FOG-1, GFI1b, and NF-E2 (Tijssen and Ghevaert, 2013). Mono-lineage production of megakaryocytes is driven by FLI1, RUNX1, and GABPα (Tijssen et al., 2011; Crispino and Weiss, 2014). The loss of GATA-1, RUNX1, and FLI1 lead to defects in megakaryocyte differentiation and polyploidization (Tijssen and Ghevaert, 2013). The polyploidization defect caused by the loss of GATA-1 can be partly attributed to the dysregulation of multiple genes. For instance, GATA-1 upregulates cyclin D1 expression, and the megakaryopoiesis impairment as a result of GATA-1 deficiency can be restored by the overexpression of cyclin D1-CDK4 in ex vivo cultured mouse megakaryocytes (Muntean et al., 2007). GATA-1 also upregulates STAT1 (Huang, et al., 2007) and CDC6 (Vilaboa et al., 2004) expression in megakaryocytes. Interestingly, estrogen increases polyploidization by elevating GATA-1 transcriptional activity (Vilaboa, et al., 2004). Furthermore, RUNX1 promotes polyploidization by silencing *MYH10* and induces an arrest of endomitosis through the upregulation of p19INK4D in later stages of differentiation (Gilles et al., 2008; Lordier et al., 2012). Recently, studies have shown that *RUNX1* deficiency causes reduced polyploidization in both in vitro and in vivo human megakaryocytes, as well as in a rhesus macaque model (Bluteau et al., 2012; Lee et al., 2023a; Lee et al., 2023b). FLI1 also regulates MYH10 and possibly p19INK4D expression levels (Antony-Debre et al., 2012). RUNX1 and FLI1 not only regulate the transition from mitosis to endomitosis but also couple the arrest in DNA replication to megakaryocyte terminal differentiation (Huang et al., 2009a). SCL/TAL1 has been

shown to affect polyploidization by regulating p21CIP1/WAF1 expression (Chagraoui et al., 2011). The role of NF-E2, a transcriptional target of GATA-1, in megakaryocyte polyploidization is more complex. *Nfe2* knockout megakaryocytes fail to undergo proplatelet formation but exhibit normal ploidy, which causes severe thrombocytopenia in mice (Shivdasani et al., 1995). On the other hand, NF-E2 can induce polyploidization of cell lines and reprogram nonhematopoietic cells into polyploid megakaryocytes (Kobayashi et al., 1998; Ono et al., 2012). NF-E2 also co-regulates with FLI-1 and RUNX1 in late megakaryocyte differentiation (Zang et al., 2016). Indeed, these transcription factors collaborate in megakaryocyte differentiation and polyploidization. Genome-wide analyses have demonstrated combinatorial interactions among GATA-1, GATA-2, RUNX1, FLI1, and TAL1 in the early stages of megakaryopoiesis and cooperation among RUNX1, FLI1, and NF-E2 at later stages (Tijssen, et al., 2011; Tijssen and Ghevaert, 2013).

GATA2, EZH2, and aryl hydrocarbon receptor (AHR) play critical roles in HSC biology and also regulate megakaryocyte polyploidization. Knockdown of *Gata2* impairs the polyploidization of megakaryocytes in vitro, and GATA2 promotes polyploidization of megakaryocytes in part by repressing the expression of myeloid transcription factors like PU.1 and C/EBPα (Huang et al., 2009b). A recent ex vitro study demonstrates that EZH2 inhibition decreases the mean ploidy of megakaryocytes derived from human cord blood $CD34^+$ cells (Mazzi et al., 2021). Specifically, EZH2 contributes to the effect of TPO on megakaryocyte commitment and permits normal polyploidization at the late stages of megakaryocyte differentiation by regulating *CDKN1A* and *CDKN2D* (Mazzi, et al., 2021). *Ahr*-null mice exhibit a decrease in the percentage of high ploidy megakaryocytes (>16N), likely due to the downregulation of HES1, a regulator of Notch signaling (Lindsey and Papoutsakis, 2011).

Some ubiquitous transcription factors are also involved in megakaryocyte polyploidization. Specifically, the MKL1(MAL)/SRF complex plays a crucial role in megakaryocyte maturation by regulating the actomyosin cytoskeleton (Cheng et al., 2009; Gilles et al., 2009; Ragu et al., 2010; Elagib et al., 2022; Reed et al., 2022). Its transcriptional activity is regulated by actin polymerization through its cellular localization, connecting cytoskeleton remodeling to transcription (Cheng, et al., 2009; Gilles, et al., 2009; Smith et al., 2013). MKL1 downregulates GEF-H1 (*ARHGEF2*) and promotes megakaryocyte polyploidization in mouse models, possibly by inducing the transition from mitosis to endomitosis (Gao et al., 2012; Reed, et al., 2022). ECT2 level reduces in higher ploidy (>4N) megakaryocytes to regulate further polyploidization (Gao, et al., 2012). Interestingly, RUNX1 and FLI1 bind to the *ARHGEF2* gene and may also regulate its expression (Tijssen, et al., 2011).

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### 3.1.3 Cell cycle regulators

Multiple cell cycle regulators are involved in megakaryocyte differentiation and polyploidization. Megakaryocytes express high levels of cyclins D1/D3, which elevate megakaryocyte number/ploidy but not platelet production (Wang et al., 1995; Sun et al., 2001). Conversely, cyclin E is essential for endomitosis as its overexpression increases megakaryocyte ploidy up to a modal 32N by regulating the pre-replication complex protein minichromosomal maintenance factor (MCM) and CDC6 (Bermejo et al., 2002; Geng et al., 2003; Eliades, et al., 2010). CDC6 overexpression in megakaryocyte cell lines similarly elevates ploidy (Bermejo, et al., 2002). Interestingly, cyclin B1 is present in megakaryocytes and undergoes conserved timing degradation during endomitosis (Zhang et al., 1996; Vitrat et al., 1998; Roy et al., 2001).

Megakaryocytes also express CDK inhibitors (CkIs) of the Cip/Kip (CDKN1) and INK4 (CDKN2) families, blocking G1/S progression. The role of INK4 family members during endomitosis remains unclear. One study found elevated p15INK4B expression in $CD41^+$ cells derived from human $CD34^+$ cells (Teofili et al., 2001), while another report showed decreased p16 expression during megakaryopoiesis (Furukawa et al., 2000). While all Cip/Kip family members (p21CIP1/WAF1, p27KIP1, p57KIP2) are present (Taniguchi et al., 1999), only p19INK4D expression correlates positively with polyploidization (Raslova et al., 2007). P19INK4D uniquely impacts basal polyploidization and megakaryocyte maturation, as p19INK4D deficiency leads to an increase of the mean ploidy, both in human megakaryocytes in vitro and mouse megakaryocytes in vivo (Gilles, et al., 2008). Although p21 overexpression inhibits polyploidization (Kikuchi, et al., 1997), p21 deletion in mice

exerts no effect on polyploidization (Baccini et al., 2001). Furthermore, the loss of p53 in mice and cultured human megakaryocytes is associated with increased megakaryocyte ploidy levels, and this effect is exacerbated under stress conditions (Apostolidis et al., 2012; Roy et al., 2016b). P16 acts as an inhibitor of polyploidization by blocking the cyclin D-Cdk4/6 complex, and its overexpression leads to the inhibition of endomitosis in ex vivo cultured mouse megakaryocytes (Muntean, et al., 2007).

Chromosome passenger proteins may regulate endomitosis due to their functions in metaphase/anaphase, cytokinesis, and abscission. Aurora A inhibition enhances polyploidization and differentiation in acute megakaryoblastic leukemia (Krause and Crispino, 2013). However, Aurora kinase A is necessary for mitosis but dispensable for endomitosis in normal megakaryopoiesis (Goldenson et al., 2015). Although Aurora kinase B localizes appropriately throughout endomitosis, it is non-essential for polyploidization (Lordier et al., 2010). Similarly, the inhibition of Polo-like kinase 1 (PLK1) causes polyploidization in the acute megakaryoblastic leukemia cell line (Wen et al., 2012), whereas *Plk1* knockout in mice induces megakaryocyte cell cycle arrest as well as spindle checkpoint activation (Trakala et al., 2015a). *Cdc20*-null causes the failure of mitotic exit leading to death and thrombocytopenia in mouse megakaryocytes (Trakala et al., 2015b). Interestingly, *Cdk1*-knockout does not inhibit polyploidization but causes endocycle for polyploidization in mouse megakaryocytes (Trakala, et al., 2015b).

### 3.2 Hepatocytes

Hepatocyte polyploidization during development has been studied almost exclusively in rodent models (Guidotti, et al., 2003; Margall-Ducos et al., 2007; Celton-Morizur et al., 2009). While all hepatocytes are diploid at birth, postnatal liver growth leads to the development of hepatocytes with varying ploidy levels, including diploid, tetraploid, and octoploid. The ploidy of a hepatocyte is determined by both its nuclear ploidy (diploid, tetraploid, octoploid) and the number of nuclei (mononucleate, binucleate). Up to 90% of hepatocytes are polyploid in adult rodents, while adult humans have approximately 30% polyploid hepatocytes (Carriere, 1967; Kudryavtsev et al., 1993; Guidotti, et al., 2003; Duncan et al., 2010; Donne, et al., 2020). In the early postnatal stage, the liver of rodents consists of diploid and proliferative hepatocytes. Diploid hepatocytes either follow a conventional cell cycle or endomitosis that involves incomplete cytokinesis (Margall-Ducos, et al., 2007). In the following cell cycle, polyploid hepatocytes could undergo incomplete or complete cytokinesis, the latter increasing the polyploidy number (Guidotti, et al., 2003). After this initial wave, the level of hepatocyte polyploidy only increases slightly throughout the organism's lifespan.

#### 3.2.1 Cytokines and signaling pathways

During the postnatal growth stage, cytokinesis failure in hepatocytes occurs during weaning, a period characterized by significant changes in feeding behavior, hormone levels, and metabolic pathways. Insulin has been identified as a crucial factor in the development of binucleate tetraploid hepatocytes (Celton-Morizur et al. ; Celton-Morizur, et al., 2009). The transition from suckling milk to taking solid food in rodents causes an increase in insulin levels. Increased insulin activates the PI3K/AKT pathway and results in the delocalization of RhoA and other essential cytokinesis effectors, which prevents the formation of cleavage furrows and ingression (Celton-Morizur, et al., 2009). The exact mechanism linking insulin and RhoA impairment is not fully understood. In rodents, reduced insulin signaling significantly decreases the production of binucleate progenies, while repeated insulin injections promote the generation of polyploid hepatocytes (Celton-Morizur, et al., 2009). The PI3K/AKT pathway is the primary downstream pathway that mediates the effects of insulin in these conditions. Multiple studies have revealed that PI3K-AKT regulates actin cytoskeleton polarization and reorganization during processes such as cell migration and invasion. In dividing hepatocytes, inhibiting AKT activity results in proper actin reorganization during mitosis and corrects RhoA localization at the division site, which are crucial for successful cytokinesis (Celton-Morizur, et al., 2009). PTEN is a negative regulator of the PI3K/AKT signaling pathway. Several studies have verified that *Pten*-deficient mice hepatocytes show in-

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creased ploidy in vivo (Gentric et al., 2015; Moreno et al., 2022).

Several other pathways have been found to negatively regulate polyploidization. Deficiencies in liver kinase B1 (LKB1) lead to hyperpolyploidy in mouse hepatocytes (Maillet et al., 2018). Mice lacking the E3 ligase S-phase kinase-associated protein 2 (SKP2) accumulate p27, resulting in extensive liver polyploidization (Minamishima et al., 2002). Interestingly, the Hippo pathway also regulates hepatocyte polyploidy through SKP2 (Zhang et al., 2017). The activation of Yes-associated protein (YAP) causes AKT phosphorylation, leading to SKP2 cytoplasmic retention and, consequently, p27 accumulation in the nucleus (Zhang, et al., 2017). Although previous studies in cell lines demonstrated the role of the Hippo pathway in preventing the cell cycle progression of tetraploids by activating p53, YAP activation in the liver results in increased ploidy independent of p53 (Ganem et al., 2014; Zhang, et al., 2017). In addition, dysregulation of the degradation of the transcription factor FOXO1/3 (forkhead box O1/3) results in hepatic hyperploidy (Zhang, et al., 2017). Hepatocyte-specific *FoxO3*-knockout reduced polyploidization of hepatocytes in a mouse model (Liang et al., 2022). In addition, WNT signaling inhibits binucleation and polyploidization of hepatocytes in an E2F7/8-dependent manner both in a mouse model and a human hepatocyte organoid model (Jin et al., 2022; Darmasaputra et al., 2024).

3.2.2 Transcription factors and cell cycle regulators

E2F transcription factors have been found to play important roles in the regulation of polyploidization during liver development (Conner et al., 2003; Chen et al., 2012; Pandit et al., 2012; Sladky et al., 2020a). This family of transcription factors consists of both activators (E2F1, E2F2, and E2F3) and repressors (E2F4, E2F5, and E2F6), as well as two atypical repressors (E2F7 and E2F8). Interestingly, the levels of E2F1-6 remain low throughout the lifespan of mice, while E2F7 and E2F8 are highly expressed during the initial 7 weeks after birth (Pandit, et al., 2012). This pattern coincides with liver polyploidization. Deficiencies in these atypical repressors result in a significant increase in diploid hepatocytes, possibly by way of dysregulating genes involving cytokinesis such as *Ect2*, *Mklp1*, and *Racgap1* (Margall-Ducos, et al., 2007). This is particularly interesting as these proteins are known to play a crucial role in the communication between the spindle and the cortex during hepatocyte polyploidization, prevent the formation of a functional contractile actomyosin ring, and ultimately lead to impaired cytokinesis (Margall-Ducos, et al., 2007). On the other hand, activators E2F1-3 initiate transcription of their target genes, including S-phase cyclins E and A, various cytokinesis effectors, and their antagonists E2F7 and E2F8 that, in turn, repress the same set of target genes (Conner, et al., 2003; Chen, et al., 2012; Pandit, et al., 2012; Bertoli et al., 2013). This feedback loop between E2F1 and E2F7/8 results in oscillating protein levels throughout the cell cycle. In mice, E2F1 deficiency alone leads to an increase in hepatocyte ploidy, while overexpression of E2F1 results in hypoploidy (Conner, et al., 2003; Chen, et al., 2012; Pandit, et al., 2012).

Deficiencies in multiple cell cycle regulator genes, including *Cdk1* (Diril et al., 2012), *Trp53* (Sheahan et al., 2004; Kurinna et al., 2013), *Cdkn1a* (encoding p21)(Wu et al., 1996; Sheahan, et al., 2004), *cMyc* (Baena et al., 2005), *Ccne* (encoding cyclin E) (Nevzorova, et al., 2009), *Birc5* (encoding survivin)(Li et al., 2013), *Ssu72* (Kim et al., 2016), *Stk3* (Mst1/2) (Zhang, et al., 2017), *Tgfb1* (De Santis Puzzonia et al., 2016), *Skp2* (Minamishima, et al., 2002; Zhang, et al., 2017), and *Rb1* (Sheahan, et al., 2004; Mayhew et al., 2005), have been shown to affect ploidy levels in hepatocytes in various mouse models. For instance, *Cdk1*-deficient hepatocytes exhibit enlarged nuclei with increased ploidy (Diril, et al., 2012), indicating that endoreduplication has occurred without cell division. Cyclin E1 and E2 have distinct and non-redundant functions in hepatocyte S phase entry and polyploidy during liver regeneration, with cyclin E1 promoting and cyclin E2 repressing polyploidization (Nevzorova, et al., 2009).

3.3.3 Additional regulators

Anillin (Anln) is required for effective hepatocyte division (Zhang et al., 2018a; Zhang, et al., 2018b). *Anln*-deficient cells progress through metaphase but are unable to form or ingress the cleavage furrow in ana-

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phase/telophase, leading to increased polyploidy (Zhang, et al., 2018a). Interestingly, microRNAs (miRNAs) have also been identified as new regulators of hepatocyte polyploidy (Hsu et al., 2016). miR-122, in particular, exhibits differential expression during postnatal development and mice lacking miR-122 showed a significant decrease in the number of polyploid hepatocytes (Hsu, et al., 2016). miR-122 directly targets and inhibits pro-cytokinesis factors during liver development, and reduces RhoA and CUX1 transcriptional levels, the latter being a transcriptional activator of ECT2 and central spindlin (Hsu, et al., 2016).

## 4 Endomitosis as a variant of mitosis in megakaryocytes and hepatocytes

Polyploid megakaryocytes can enter into mitosis and exhibit the formation of a complex multipolar spindle (with 2N poles between 2N and 4N, four poles between 4N and 8N, and so on) and an asymmetrical segregation of chromosomes towards the different poles (Vitrat, et al., 1998; Roy, et al., 2001; Geddis et al., 2007; Papadantonakis et al., 2008). Endomitosis was once believed to be an incomplete mitosis that skips telophase and cytokinesis (Nagata, et al., 1997; Vitrat, et al., 1998). In fact, endomitotic cells could proceed to telophase and the two daughter cells are nearly separated with a seemingly normal midzone and the formation of a cleavage furrow, particularly in transition from 2N to 4N (Geddis, et al., 2007). However, this furrow regresses rapidly in endomitotic megakaryocytes, and the two daughter cells fuse together (Geddis, et al., 2007). The abnormalities in furrow ingression are more significant in higher ploidy, leading to minimal cell elongation and chromosome segregation (Papadantonakis, et al., 2008). Significantly prolonged M phase has been observed in endomitotic megakaryocytes (Trakala, et al., 2015a), which may consequently lead to cell cycle exit without cytokinesis. In rodent hepatocytes, disruption of canonical cytokinesis resulting from the absence of actomyosin ring formation, and impaired actin organization during anaphase-telophase has been observed (Celton-Morizur and Desdouets, 2010; Fortier et al., 2017). Consequently, essential microtubule-associated proteins, such as Aurora B, PRC1, MgcRacGAP, and PLK1 fail to localize properly, impeding RhoA concentration and contractile ring formation at the division site. Disorganization of microtubules during incomplete cytokinesis impairs molecular signaling to the equatorial cortex (Margall-Ducos, et al., 2007). Active RhoA concentrates equatorially during normal cytokinesis but remains diffuse throughout the center of cells undergoing cytokinesis failure (Margall-Ducos, et al., 2007). These observations demonstrate that improper regulation of RhoA signaling stemming from disorganized microtubules during incomplete cytokinesis ultimately leads to the production of polyploid hepatocytes. Similar to observations in megakaryocytes, diploid hepatocytes are found to enter and complete the cell cycle more rapidly than polyploid hepatocytes (Wilkinson et al., 2019). Differences are also noted among polyploid hepatocytes, with tetraploids initiating cell cycle progression at a faster pace relative to octoploids, suggesting that cell cycle regulation may exhibit subtle variances between diploidy and polyploidy (Wilkinson, et al., 2019). Notably, recent live-imaging analyses of fetal tissue-derived human hepatocyte organoids reveal that human hepatocytes undergoing endomitosis inhibit cell division during a late step in cytokinesis (Darmasaputra, et al., 2024). More similar to megakaryocytes than rodent hepatocytes, endomitotic human hepatocytes exhibit normal cytokinetic furrow ingression; however, the ingressed membrane detaches from the midbody during late cytokinesis. Proteins involved in anchoring the midbody to the cell cortex are present but lose their association with the cell membrane during endomitotic furrow regression (Darmasaputra, et al., 2024). These observations imply that endomitosis exhibits some typical characteristics of mitosis. Endomitosis essentially may be a variant of mitosis.

Endomitotic megakaryocytes and hepatocytes exhibit notable distinctions, particularly in the number of nuclei (Fig. 1). The majority of polyploid megakaryocytes are large, mononucleated cells, while polyploid hepatocytes can be either mononucleated or binucleated. It has been hypothesized that the progression of endomitosis may determine the mono- or binucleation status: cytokinesis failure at late M phase results in binucleation, whereas exiting M phase at the early stage leads to mononucleation (Darmasaputra, et al., 2024). In fact, megakaryocytes display significant furrow ingression and regression during the 2N to 4N transition, and a

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substantial proportion of 4N megakaryocytes are truly binucleated (Lordier et al., 2008; Leysi-Derilou et al., 2010), indicating a late cytokinesis defect. In contrast, most of the higher ploidy megakaryocytes are mononucleated and also show measurable furrowing followed by regression (Lordier, et al., 2008; Leysi-Derilou, et al., 2010). In sharp contrast, rodent endomitotic hepatocytes lack a central spindle, cleavage furrow ingression, and anaphase cell elongation (Guidotti et al., 2003; Margall-Ducos et al., 2007), suggesting that endomitosis in rodent hepatocytes corresponds to an early cytokinesis failure, yet still produces binucleated polyploid cells. Moreover, the formation of mononucleation in megakaryocytes has been proposed to be a karyokinesis defect with the persistent presence of nucleoplasmic bridges (Lordier et al., 2012b), which is not observed in hepatocyte endomitosis (Margall-Ducos et al., 2007; Darmasaputra et al., 2024). Indeed, during liver growth, the mononucleated polyploid hepatocytes are essentially derived from the cell division of binucleated polyploid hepatocytes (Donne, et al., 2020). Whether cells become mononucleated or binucleated is not critical for endomitosis leading to polyploidy, while there might be functional distinctions between different nucleations (Katsuda et al., 2020; Richter et al., 2021; Dehn and Losick, 2022; Van Rijnberk et al., 2022). Despite these differences, endomitotic cells exhibit remarkable similarities in their transition from diploidy to polyploidy (Ravid et al., 2002; Guidotti et al., 2003; Ganem et al., 2014; Gentric and Desdouets, 2014; Mazzi et al., 2018; Ovrebo and Edgar, 2018).

In general, commonalities and specificities of endomitosis exist in megakaryocytes and hepatocytes. Undoubtedly, endomitosis is a fundamental biological process rather than merely a developmentally-coupled phenomenon. Whether cells decide to divide or not involves cell fate decision during the cell cycle. The fundamental mechanisms responsible for this cell fate decision involve elegant regulation of M phase and remain to be addressed. How endomitotic cells manipulate mitosis to achieve this in megakaryocytes and hepatocytes warrants further clarification, which may reveal commonalities and specificities.

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## 5 Mechanisms rewiring mitosis towards endomitosis for polyploidization

As summarized above, most studies on polyploidization regulators are tightly coupled with development, organogenesis, and cell differentiation. However, these studies have not addressed the essence of endomitosis, a fundamental biological process involving cell cycle regulation. Several key events involving mitosis phase have to be modified for cells to progress to this cell fate (Fig. 2).

### 5.1 Endomitosis requires functional mechanisms regulating M phase entry and exit

CDK1 is known to be a critical regulator of M phase entry and is also indispensable for endomitosis (Santamaria et al., 2007). An initial study showed that cyclin B1, the CDK1 activity regulator, expression was undetectable or decreased in megakaryocytes (Zhang, et al., 1996). However, later studies confirmed that both cyclin B1 and CDK1 are expressed in endomitotic megakaryocytes and are normally degraded by anaphase-promoting complex or cyclosome (APC/C) along with its co-activators, CDC20 and CDH1 (Vitrat, et al., 1998; Roy, et al., 2001). *Cdk1*-null megakaryocytes and hepatocytes both fail to enter M phase and proceed to endocycle rather than endomitosis (Diril, et al., 2012; Trakala, et al., 2015b).

As the activation of the Spindle Assembly Checkpoint (SAC) is imperative for mitosis, where CDK1/cyclin B serves as a linchpin, the SAC may play a crucial role in endomitosis as well. CDK1 contributes to the SAC, which ensures the integrity of the chromosome and allows the M phase to proceed to anaphase (Bentley et al., 2007; Chen et al., 2008; Alfonso-Perez et al., 2019). In fact, the SAC is present and functions in megakaryocytes undergoing endomitosis (Roy, et al., 2001; Trakala, et al., 2015a). Typically, the SAC will be activated and arrest the mitotic cells in metaphase until all the chromosomes are fully attached (Musacchio and Salmon, 2007). However, failure to satisfy the SAC sometimes causes cells to slip out of the arrest, a phenomenon called mitotic slippage (Sinha et al., 2019). In this context, progressive cyclin B1 degradation allows

cells to escape mitosis and reach the G1 phase, even when the SAC is active (Brito and Rieder, 2006). Furthermore, reduced SAC activity is suggested to promote polyploidization in megakaryocytes (Wang et al., 2004; Brito and Rieder, 2006; Trakala, et al., 2015a). The level of SAC activity in endomitotic cells and its impact on polyploidization is an attractive question to explore.

PLK1 is believed to involve these processes. In megakaryocytes undergoing endomitosis, sister chromatid separation appears to occur asymmetrically toward opposite poles rather than symmetrically, with chromosomes clustering around each pole into rings, ultimately forming multilobed megakaryocytes (Roy, et al., 2001). This asymmetrical separation to multiple poles suggests that the SAC allows megakaryocyte chromosomes to attach complexly to multiple spindle poles. This is altered in *Plk1*-deficient megakaryocytes. With a deficiency of PLK1, all chromosomes form single or a few rings around the sole or minority of poles, consistent with megakaryocytes in vivo having single or fewer lobes (Trakala, et al., 2015a). These spindle assembly defects during formation could lead to SAC-dependent mitotic arrests, which typically trigger cell death (Trakala, et al., 2015a; Sinha, et al., 2019).

APC/C targeting cyclin B for its degradation also contributes to endomitosis, as a low level of cyclin B is a key factor for M phase exit. The APC/C-CDC20 complex is an E3-ubiquitin ligase, which is essential for exiting mitosis by triggering the degradation of the CDK1 activator cyclin B1 (Peters, 2006). CDC20 ablation in mice does not have any effect on interphase or mitotic entry, but these *Cdc20*-deficient cells are trapped in mitosis due to the absence of APC/C-CDC20 activity (Manchado et al., 2010). Deletion of *Cdc20* in mice causes the accumulation of abnormal small megakaryocytes that display hyper-condensed chromosomes and mitotic arrest (Trakala, et al., 2015b). Over 80% of *Cdc20*-null megakaryocytes are arrested in metaphase and cannot exit from M phase (Trakala, et al., 2015b). In late mitosis and G1, APC/C associates with CDH1, which continues to keep mitotic cyclins low until the next G2/M, and this event is also conserved in endomitotic megakaryocytes (Roy, et al., 2001; Peters, 2006).

In the conserved mitotic exit mechanism, the orchestration of kinases and phosphatases plays a pivotal role (Wurzenberger and Gerlich, 2011; Manic et al., 2017). The Aurora kinases A and B are both proved to be dispensable for megakaryocyte polyploidization (Lordier, et al., 2010; Goldenson, et al., 2015). However, the inhibition of Aurora B profoundly modifies the endomitotic process by inducing a missegregation of chromosomes and a mitotic failure in anaphase (Lordier, et al., 2010). This defect may be related to the abnormal localization of Aurora B due to the inhibition of its kinase activity. Clarifying the role of other kinases and phosphatases in regulating endomitotic entry and exit will advance our understanding of endomitosis.

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### 5.2 Incomplete cytokinesis is indispensable

Endomitotic cells need to initiate and exit M phase without cytokinesis due to cytokinesis failure or mitotic slippage, as completing cytokinesis would result in the production of either two faithfully separated daughter cells or two aneuploid cells rather than a polyploid cell (Leysi-Derilou, et al., 2010; Fededa and Gerlich, 2012; Lacroix and Maddox, 2012). Cytokinesis failure can arise through defects in any of the four stages of the process: i) positioning of the division plane, ii) ingression of the cleavage furrow, iii) formation of the midbody, and iv) abscission (Wang, et al., 2017). Although cytokinesis is inhibited at different stages of M phase in megakaryocytes (Geddis et al., 2007; Gao et al., 2012), cardiomyocytes (Engel et al., 2006; Leone et al., 2018), and hepatocytes (Guidotti et al., 2003; Celton-Morizur et al., 2009; Margall-Ducos et al., 2007; Pandit et al., 2012), the downregulation or inhibition of key cytokinesis regulators seems to be a common feature.

The absence of active RhoA in the cleavage furrow seems to be the most critical factor, which can be attributed to either the abnormal localization of RhoA or a defect in RhoA activation. The RhoA pathway plays a key role in cleavage furrow formation and ingression by facilitating actomyosin ring assembly, which generates contractile forces for abscission (Vainchenker et al., 2021). RhoA activation at the cleavage furrow induces localized actin polymerization and myosin II accumulation/activation, allowing furrow progression and abscission. This is mediated via formin/profilin for F-actin polymerization, as well as Rho kinase (ROCK) and

citron kinase phosphorylating myosin light chain 2 (MLC2) for myosin activation. In megakaryocyte endomitosis, there is defective accumulation of myosin and F-actin, despite residual myosin activity remaining (Geddis and Kaushansky, 2006; Lordier, et al., 2008). The RhoA/ ROCK pathway is downregulated during the process of megakaryocyte endomitosis (Lordier, et al., 2008; Gao, et al., 2012; Avanzi et al., 2014). However, megakaryocytes from mice conditionally lacking RhoA or Cdc42 do not show impaired megakaryocyte endomitosis (Heib et al., 2021), suggesting a critical role of RhoA/Cdc42 in mitosis but not endomitosis, similar to Survivin (Wen et al., 2009). Although RhoA properly localizes in the cleavage furrows, particularly during the transition from 2N to 4N, there is a defect in RhoA activation in megakaryocytes (Geddis and Kaushansky, 2006; Lordier, et al., 2008). Consistently, incomplete cytokinesis in hepatocyte endomitosis is also characterized by an absence of active RhoA localization at the putative cleavage plane (Margall-Ducos, et al., 2007). RhoA activity relies on its guanine nucleotide exchange factors (GEFs) like GEF-H1 and ECT2, which localize to cleavage furrows. GEF-H1 downregulation coincides with 2N to 4N transitions, while ECT2 markedly decreases at higher ploidy stages during megakaryocyte polyploidization (Gao, et al., 2012). The downregulation of ECT2 in both hepatocytes and high-ploidy (>4N) megakaryocytes may explain the lack of significant ingression of the cleavage furrow during cytokinesis failure in both cell types (Papadantonakis, et al., 2008; Gao, et al., 2012; Pandit, et al., 2012). During megakaryocyte differentiation, two types of myosin II are synthesized: myosin IIA (MYH9) and myosin IIB (MYH10). However, in megakaryocyte precursors, myosin IIB is almost the only myosin II to be recruited at the cleavage furrow due to the structural differences between myosin IIA and IIB (Badirou et al., 2014; Roy et al., 2016a). Furthermore, during megakaryocyte endomitosis, the transcriptional downregulation of GEF-H1 and MYH10 leads to a marked defect of contractile forces in the cleavage furrow, potentially explaining the abortive cytokinesis (Gao, et al., 2012; Lordier, et al., 2012; Badirou, et al., 2014). Additional mitotic spindle components may influence endomitosis, such as downregulated microtubule-destabilizing Stathmin (Iancu-Rubin et al., 2005; Iancu-Rubin et al., 2011).

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### 5.3 Tetraploidy is a critical stage for endomitosis

Tetraploid cells at G2/M phase may proceed to cytokinesis for the next round of the cell cycle, whereas a prolonged G2/M phase causes apoptosis. In rare conditions, a cell may bypass cell division and transit to G1 for the next cell cycle, an event termed whole-genome doubling (WGD). Unscheduled WGD cells typically face replicative stress and DNA damage and exhibit genomic instability due to unbalanced chromosome segregation in subsequent mitosis (Quinton et al., 2021; Gemble et al., 2022; Lambuta et al., 2023). Typically, unscheduled WGD cells are arrested in the subsequent G1 phase due to the activation of p53 signaling and the Hippo pathway, a mechanism known as the "tetraploidy checkpoint," and the associated cell death is termed "mitotic catastrophe" (Margolis et al., 2003; Vitale et al., 2011; Ganem, et al., 2014). p53 activation prevents cells from re-entering the cell cycle, leading to senescence (Notterman et al., 1998; Andreassen et al., 2001; Aylon and Oren, 2011). Furthermore, the p53 and Hippo pathways are interconnected via LATS2 (a Hippo effector), ensuring the post-WGD G1 arrest (Aylon et al., 2006; Ganem et al., 2014). The tetraploidy checkpoint is recognized as a significant barrier to WGD (Margolis et al., 2003; Ganem et al., 2014). Additionally, extra centrosomes may influence WGD. The PIDDosome acts as a sensor for supernumerary centrosomes, a hallmark of tetraploid cells (Fava et al., 2017). The PIDDosome, a multiprotein complex that activates caspase-2 (CASP2), includes the death domain (DD) containing proteins p53-induced DD protein 1 (PIDD1) and CRADD (also known as RAIDD). The accumulation of extra centrosomes triggers the PIDDosome signaling cascade, resulting in cell cycle arrest (Fava et al., 2017). The Hippo pathway also senses extra centrosomes (Sanz-Gómez, et al., 2023). The presence of supernumerary centrosomes in RPE1 cells inhibits RhoA GTPase activity and activates Rac1 (Ganem, et al., 2014). This modification of the cytoskeleton GTPases RhoA and Rac1 activates the Hippo pathway as a protective mechanism (Yu et al., 2012; Ganem et al., 2014). In fact, p53, LATS2, and the PIDDosome are considered components of the "tetraploidy checkpoint" that prevents tetraploid cells from re-entering the cell cycle, as the inactivation of any of these genes reverses the G1 arrest (Aylon et al., 2006;

Aylon and Oren, 2011; Ganem et al., 2014; Sladky et al., 2020).

Endomitotic cells must overcome tetraploidy restrictions to transit from diploidy to polyploidy. Consequently, it is plausible that hepatocytes and megakaryocytes have a unique ability to bypass the tetraploidy checkpoint and proceed to polyploidization (Roy et al., 2016b; Zhang et al., 2017). Although the precise mechanism remains largely unknown, low RhoA activity during megakaryocyte polyploidization fails to activate the Hippo pathway ex vivo, resulting in reduced levels of phosphorylated YAP but a high overall level of total YAP protein (Roy et al., 2016). However, *YAP* knockdown does not affect the ploidy of ex vivo cultured human megakaryocytes (Roy et al., 2016). In contrast, tetraploid hepatocytes in mice have been shown to activate the Hippo pathway in vivo (Ganem et al., 2014). Activation of YAP/TAZ signaling, either by inhibiting the Hippo pathway or by direct YAP overexpression in vivo, leads to increased ploidy in hepatocytes (Lee et al., 2016; Zhang et al., 2017). Specifically, inhibition of the Hippo pathway in hepatocytes in vivo results in the accumulation of supernumerary centrosomes and cytokinesis failure (Zhang et al., 2017).

p53 is often referred to as the "guardian of the ploidy" due to its role in preventing WGD cells from re-entering the cell cycle by arresting them in the G1 phase and eventually triggering a non-proliferative state (Aylon and Oren, 2011; Cross et al., 1995; Andreassen et al., 2001). To bypass the tetraploid barrier, endomitotic hepatocytes regulate ploidy via the PIDDosome-p53 pathway (Fava et al., 2017; Sladky et al., 2021; Sladky et al., 2022). Unlike typical cells that undergo PIDDosome-mediated apoptosis following multiple rounds of centrosome duplication, hepatocytes downregulate CASP2 via E2F7 and E2F8 to attain a physiologically normal polyploid state (Sladky et al., 2020a). The E2F transcriptional circuit further promotes polyploidization by downregulating cytokinesis machinery. PIDDosome-deficient mice exhibit significantly increased hepatocyte ploidy levels. Similarly, the loss of p53 in mice and *p53* knockdown in cultured human megakaryocytes are both associated with increased megakaryocyte ploidy levels (Fuhrken et al., 2008; Apostolidis et al., 2012; Roy et al., 2016b). However, the role of the PIDDosome-p53 pathway in regulating megakaryocyte ploidy and endomitosis is still under investigation. Recent studies have shown that PIDD1 is recruited to the older centriole by the centriolar distal appendage protein ANKRD26, which is essential for PIDDosome activation in response to excess centrosomes (Burigotto et al., 2021; Evans et al., 2021). Interestingly, *ANKRD26* is typically silenced during the late stages of healthy megakaryopoiesis, and mutations in *ANKRD26* found in thrombocytopenic patients disrupt this repression (Bluteau et al., 2014; Burigotto, et al., 2021; Evans, et al., 2021). Consequently, megakaryocytes from these patients exhibit reductions in ploidy (Bluteau, et al., 2014). It is very likely that the reduction in megakaryocyte ploidy in these patients arises due to ANKRD26-mediated activation of the PIDDosome in megakaryocytes with extra centrosomes (Burigotto, et al., 2021; Evans, et al., 2021). Further research in this area may elucidate how megakaryocytes tolerate supernumerary centrosomes and naturally prevent PIDDosome activation during endomitosis.

Tetraploidy checkpoint may not be the only challenge that endomitotic cells face. A recent study indicates that significant replication stress is a major source of genetic and chromosomal instability in newly formed WGD cells (Gemble et al., 2022). Mechanistically, these defects arise from a shortage of essential proteins during the first G1/S transition after WGD induction, compromising the fidelity of DNA replication and leading to high levels of DNA damage and abnormal karyotypes (Gemble et al., 2022). Furthermore, a subsequent study suggested that the oncogenic transformation of tetraploid cells is linked to this protein insufficiency (Lambuta et al., 2023). As endomitosis is a process leading to a WGD event in a physiological context, G1 arrest triggered by the tetraploidy checkpoint is suggested to be crucial for endomitotic cells to restore protein levels (Lambuta et al., 2023). An earlier study supports this hypothesis, confirming that p21 is highly expressed in hypoploid (2N and 4N) and polyploid (at least 8N) megakaryocytes derived from human $CD34^+$ cells (Baccini et al., 2001). High levels of p27 (Kip1), p16, cyclin E, and cyclin D3 were also observed in these populations, along with a hypophosphorylated form of retinoblastoma protein (Rb), indicating that most hypoploid and polyploid megakaryocytes are G1-arrested cells (Baccini et al., 2001; Besancenot et al., 2010). Thus, it appears that endomitotic cells must overcome the tetraploidy restrictions to transition from the canonical cell cycle to endomitosis while also mitigating the detrimental effects on cell fitness to achieve a stable polyploid state.

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## 6 Perspectives

Endomitosis is essentially a variant form of mitosis, and cells rewire mitosis towards endomitosis for polyploidization. Dysregulation of polyploidization is associated with multiple types of diseases. For instance, myeloproliferative neoplasms exhibit increased proliferation and decreased polyploidization in megakaryocytes, leading to thrombocythemia (Wen et al., 2015). Loss of polyploidization causes acute megakaryoblastic leukemia, and induction of polyploidization has been suggested as a novel differentiation therapy (Wen et al., 2012; Krause and Crispino, 2013). Further studies should focus on characterizing the unique features of the M phase in endomitotic cells. How tetraploid cells re-enter the G1 phase for the next round of the endomitotic cell cycle is a critical point. Recent technical developments allow us to distinguish polyploid cells in the M phase from those in the G1 phase (Sakaue-Sawano et al., 2008; Sakaue-Sawano et al., 2013). With the emergence of single-cell and nuclear sequencing (Katsuda, et al., 2020; Richter, et al., 2021), further studies may decipher the gene expression programs in polyploid cells at G1 and M phases.

Previous studies used nocodazole or other drugs to induce WGD (Gemble, et al., 2022). It cannot be ruled out that the results of these studies are add-on effects of the drug treatment (Uetake and Sluder, 2004; Wong and Stearns, 2005). Additionally, the tetraploid cells in these studies did not truly enter the endomitosis cell fate, as these cells are prone to divide in the following cell cycle rather than undergoing consecutive cycles of endomitosis to accomplish polyploidization. WGD has been observed in early and pre-malignant lesions of various tissues, and it is estimated to occur in approximately 30% of human cancers (Zack et al., 2013; Bielski et al., 2018; Quinton, et al., 2021; Zeng et al., 2023; Mckenney et al., 2024). These observations raise intriguing questions. Why do tetraploid cells originating from WGD not proceed to endomitosis? Why do megakaryocytes achieve such high ploidy via consecutive cycles of endomitosis but rarely undergo malignant transformation? Indeed, paradoxically, polyploidy has been shown to both promote and suppress tumorigenesis (Ganem et al., 2007; Zhang et al., 2018b; Zhang et al., 2019; Bou-Nader et al., 2020). The formation of tetraploid and polyploid cells in physiological contexts, such as in megakaryocytes and hepatocytes, can serve as perfect models for studying these problems. Dissecting the mechanisms underlying endomitosis is important not only for understanding physiological polyploidization in normal cells but also for understanding related diseases.

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### Author contributions

Qi-Hua HUA wrote the original manuscript. Qi-Hua HUA and Zan HUANG conceived, designed, discussed the work, and revised the manuscript. Xuechun ZHANG and Ruifeng TIAN performed the experiments and prepared the immunofluorescence images for Fig. 2. Qi-Hua HUA created Fig. 1 and processed the immunofluorescence images in Fig. 2. Zan HUANG and Zhigang SHE administered and supervised this work.

All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

### Compliance with ethics guidelines

Qi-Hua HUA, Xuechun ZHANG, Ruifeng TIAN, Zhigang SHE, and Zan HUANG declare no conflict of interest regarding the publication of this review article.

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## Figures and Figure Legends:

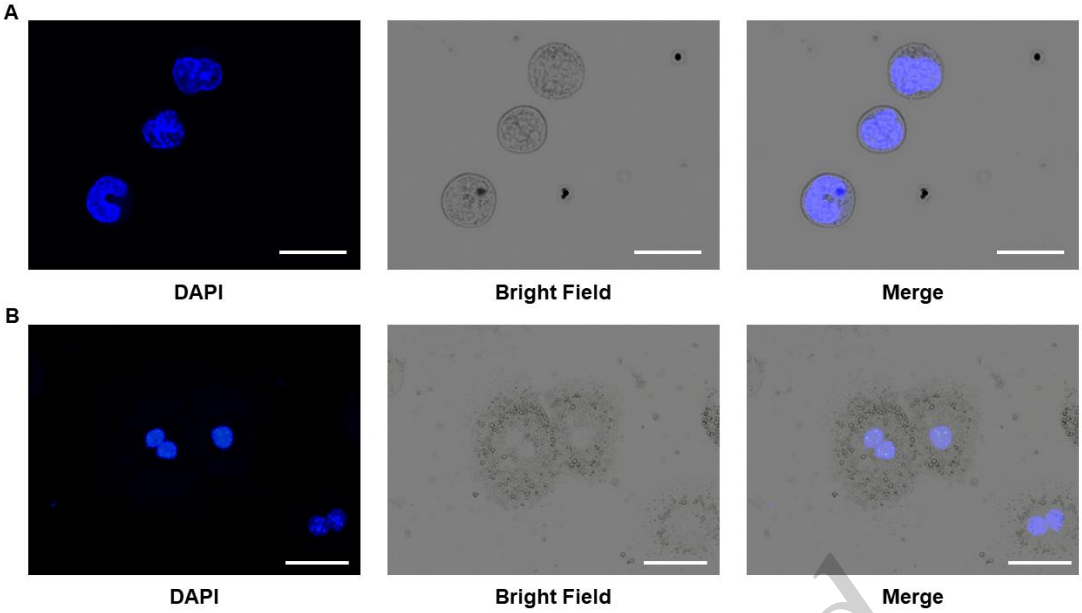


**Fig. 1 Illustration showing different nucleations of endomitosis in megakaryocytes and hepatocytes.**
The immunofluorescence images of endomitotic megakaryocytes and hepatocytes. (A) Polyploid megakaryocytes are typically mononucleated, possessing a single nucleus that is multilobulated. Nuclei of polyploid megakaryocytes stained with DAPI (left), bright field image (middle), and merged image (right). Scale bars, 15 μm. (B) Polyploid hepatocytes are mononucleated or binucleated. Nuclei of polyploid hepatocytes stained with DAPI (left), bright field image (middle), and merged image (right). Scale bars, 30 μm.

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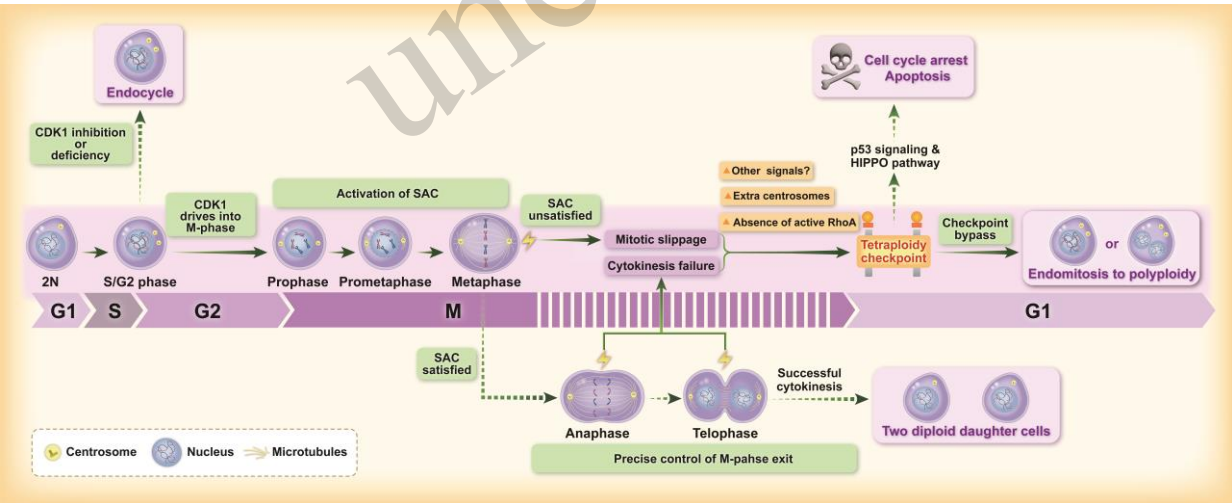


**Fig. 2 Crossroads of endomitotic cell fate decision**
Endomitotic cells can enter and exit mitosis without undergoing complete cytokinesis. During endomitosis, cells manipulate multiple critical events in the cell cycle and bypass the tetraploidy checkpoint, thereby achieving polyploidy. The solid arrows in the schematic illustration represent the canonical route of endomitosis cell fate, while the dashed arrows indicate alternative cell fates, such as endocycle, mitosis, and cell death induced by the tetraploidy checkpoint. CDK1, cyclin dependent kinase 1; SAC, spindle assembly checkpoint; RhoA, Ras homolog family member A.