

REVIEW

Polyploidy in tissue homeostasis and regeneration

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ABSTRACT

Polyploid cells, which contain multiple copies of the typically diploid genome, are widespread in plants and animals. Polyploidization can be developmentally programmed or stress induced, and arises from either cell-cell fusion or a process known as endoreplication, in which cells replicate their DNA but either fail to complete cytokinesis or to progress through M phase entirely. Polyploidization offers cells several potential fitness benefits, including the ability to increase cell size and biomass production without disrupting cell and tissue structure, and allowing improved cell longevity through higher tolerance to genomic stress and apoptotic signals. Accordingly, recent studies have uncovered crucial roles for polyploidization in compensatory cell growth during tissue regeneration in the heart, liver, epidermis and intestine. Here, we review current knowledge of the molecular pathways that generate polyploidy and discuss how polyploidization is used in tissue repair and regeneration.

KEY WORDS: Endomitosis, Endocycling, Polyploid, Wound, Healing, Hippo

Introduction

Cells are considered polyploid if they possess three or more complete copies of the haploid genome. This condition can arise either through cell-cell fusion or via a process known as endoreplication. A polyploid genome may be transmitted through the germline, thus resulting in an organism that is wholly polyploid. This condition, which is termed ‘autopolyploidy’ (see Glossary, Box 1), is common in plants, both in the wild and in crop varieties, and is believed to result from chromosome mis-segregation during meiosis. Polyploid cells may also arise from diploid cells in somatic tissues, a condition defined as ‘endopolyploidy’, which is the topic of this Review.

Endopolyploid cells are generated via endoreplication cell cycles in which cells successively replicate the genome without completing cytokinesis during mitosis (Fig. 1); such cells replicate their DNA during S phase and either revert back to a gap (G) phase, skipping mitosis completely (in the case of endocycling), or enter mitosis and fail to complete cytokinesis (in the case of endomitosis). Endoreplicating cells are known to reach ploidies greater than 200,000C (where C=total ‘chromatin’ value, as a multiple of the haploid genome; see Glossary, Box 1). This increase in genomic DNA content allows a higher transcriptional output, which can facilitate the growth of very large cells and/or enhance macromolecular secretion. Endoreplication is found in all eukaryotic kingdoms, i.e. in plants, fungi, protozoa and animals (Joubès and Chevalier, 2000; Rusch et al., 1966; Yin et al., 2010; Zielke et al., 2013). The sea hare *Aplysia* possesses giant neurons

that can reach ploidies of 260,000C, and massive ploidy has also been noted in neurons, epidermal gland cells and digestive glands in other mollusks (*Achatina*, *Helix* and *Lymnaea*) (Anisimov, 2005; Lasek and Dower, 1971; Mandrioli et al., 2010). There are also several examples in which somatic growth depends upon increases in ploidy, such as in *Caenorhabditis*, *Oikopleura* and *Drosophila* (Edgar and Orr-Weaver, 2001; Ganot and Thompson, 2002; Sulston and Horvitz, 1977). Polyploidy is also widespread in plant tissues, including tomato fruit, peanuts, maize kernels and the leaf epidermis (Joubès and Chevalier, 2000). Owing to its widespread nature and ability to sustain increased cell growth, endoreplication has been estimated to contribute significantly to biological mass in nature, possibly even more than cell proliferation (Sugimoto-Shirasu and Roberts, 2003).

In mammals, polyploidy is represented to a variable extent in many tissues, and can be induced by developmental programming, as a response to tissue injury, or by failed mitosis (mitotic slippage). Transient polyploidization is also known to occur in tumorigenesis, giving rise to aneuploid cells (see Box 2). Because polyploid cells tend to possess a higher capacity for growth (Orr-Weaver, 2015), such cells are likely to support organ growth and tissue homeostasis in the absence of mitosis. This ability has been proposed to support regeneration of the mammalian liver upon chemotoxic stress and aging (Gentric et al., 2012; Gupta, 2000), and wound healing in *Drosophila* (Losick, 2016; Losick et al., 2013, 2016). The growth of polyploid cells is thought to be fueled by their increased gene copy number, supporting increased mRNA and protein synthesis (Zhurinsky et al., 2010). Other advantages associated with polyploidy include genomic buffering against mutations, resistance to apoptosis, increased lifespan and increased metabolism. Polyploid cells also tend to be more efficient in forming protective tissue envelopes and barriers (Orr-Weaver, 2015), possibly because tissues formed by larger cells require a lower density of cell-cell junctions. In addition, because mitosis involves a reorganization of the cytoskeleton and a loss of cell adhesion, compensatory polyploidization-mediated growth may be a preferred option to replace lost cell mass, or to relieve tissue tension, in the event of tissue injury or stress. As such, a better understanding of wound-induced polyploidization may potentially spawn novel therapeutic strategies for healing tissues with poor regenerative capacity.

In this Review, we first summarize examples of polyploidy that are found in nature and then discuss the molecular mechanisms that can induce and regulate endoreplication in different organisms. Finally, we discuss recent discoveries highlighting how endocycling, endomitosis and/or cell-cell fusion can contribute to tissue homeostasis and regeneration.

Developmental endoreplication

Endoreplication has been reported in several groups of plants and animals, where it often contributes to increased cell and/or body size. In plants, endocycling is an essential aspect of normal development in many cell types (Breuer et al., 2010; Caro et al.,

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Box 1. Glossary

C ploidy values. C indicates chromatin amount or DNA content, as a multiple of the haploid genome (e.g. 4C is the chromatin amount of a diploid cell in G2 or a tetraploid cell in G1) (Brodsky et al., 1985; White, 1973a).

n (or N) ploidy values. The number of sets of chromosomes, as a multiple of the haploid genome: $1n$ is the chromosome content of a sperm or egg cell and $2n$ is the chromosomal content of a diploid cell (with two haploid sets of chromosomes – one maternal and one paternal).

Allopolyploid. An organism with more than two haploid sets of chromosomes that have been derived from two or more species by hybridization. Allopolyploidy usually arises from cell-cell fusion between gametes of two related species, where at least one gamete has a ploidy $yn > 1n$ (y =whole number) (White, 1937).

Aneuploid. A genome in which partial genomic content has been gained or lost (e.g. $yn-x$, $yn+x$), usually as a product of chromosome mis-segregation.

Autopolyploid. An organism with more than two haploid sets of chromosomes that have been derived from the same parent species. Autopolyploidy usually arises from cell-cell fusion between gametes, where at least one gamete has a ploidy $yn > 1n$ (White, 1937).

Endopolyploid. Somatic cells that are polyploid ($yC > 2C$). Endopolyploidy usually occurs as a result of endoreplication, but also includes cell-cell fusion (White, 1973b).

Euploid. Genome with an exact multiple of the haploid genome (yn).

Polytene chromosomes. Chromosomes containing multiple parallel strands of DNA.

2008; De Veylder et al., 2011; Gutierrez, 2005, 2009; Harashima and Schnittger, 2010; Sabelli and Larkins, 2009). In *Arabidopsis thaliana*, most leaf cell types enter the endocycle and reach ploidies of up to 32C, such that ploidy and cell expansion, and consequently leaf growth, are correlated (Kondorosi et al., 2000; Melaragno et al., 1993; Sugimoto-Shirasu and Roberts, 2003). In metazoans, an example of consistent regulation of cell size and patterning by polyploid cells has been documented in the urochordate *Oikopleura dioica*, a pan-global species of marine zooplankton. This marine organism loses most mitotic features a few hours after hatching, as most cells transit to developmentally controlled endocycles, giving rise to ploidies ranging from 4C to 1300C, with cell sizes proportional to their ploidy (Campsteijn et al., 2012; Ganot and Thompson, 2002). As a result, *Oikopleura* grows 10-fold during its 1-week life cycle.

Endoreplication is also common in insects and has been extensively characterized in the fruit fly *Drosophila melanogaster* (Edgar and Orr-Weaver, 2001; Edgar et al., 2014; Fox and Duronio, 2013; Fox et al., 2010; Swanson et al., 2015; Zielke et al., 2013). Most differentiated larval tissues of *Drosophila* develop through endocycling, which has been studied in the salivary glands, fat body, gut, renal tubes, trachea and epidermis (Smith and Orr-Weaver, 1991). Mitotic proliferation is mostly retained in undifferentiated progenitor and stem cells, including imaginal discs and adult midgut progenitors of the larval gut. Several cell types in the adult fly are also polyploid, including neurons, sensory bristles, gut enterocytes and ovarian nurse and follicle cells, with ploidies ranging from 8C to 2000C (Audibert et al., 2005; Hammond and Laird, 1985; Unhavaithaya and Orr-Weaver, 2012). Endocycling is also required for increased growth in these cell types, and is therefore necessary to sustain normal cellular

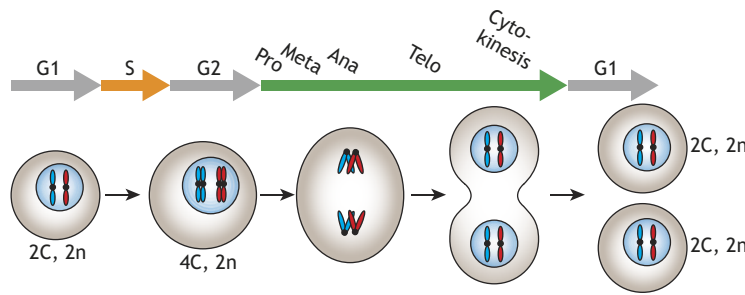
functions. For example, decreased ploidy of ovarian nurse cells causes female sterility, because endocycling is required for normal oocyte development (Lilly and Spradling, 1996; Maines et al., 2004).

Developmental endopolyploidy has also been reported in fish (Mandrioli et al., 2010), mice and humans, and likely occurs in most mammals. In fact, it has been estimated that mammalian tissues are generally composed of up to 20% polyploid cells (Biesterfeld et al., 1994), although some human tissues have been estimated to contain ~50% and ~70% polyploid cells (Gandarillas and Freije, 2014; Mollova et al., 2013). Vertebrate cells known to undergo developmental endoreplication include placental trophoblast giant cells (TGCs), endometrial stromal cells (Qi et al., 2015), cardiac myocytes (Mollova et al., 2013; Soonpaa et al., 1996), hepatocytes (Gupta, 2000), megakaryocytes (Ravid et al., 2002; Trakala et al., 2015), keratinocytes (Gandarillas, 2012; Gandarillas and Freije, 2014), epicardial cells and vascular smooth muscle cells (Cao et al., 2017; McCrann et al., 2008). In addition, mammary epithelial cells are bi-nucleate (Rios et al., 2016), but whether this polyploidy arises from cell-cell fusion or endoreplication is not clear. Thus, polyploidy may be of importance whether one is looking at cancer, aging, tissue homeostasis or wound healing.

Molecular control of endoreplication and polyploidization

Polyploidization and the switch from mitosis to endoreplication often take place as part of a developmental program that involves the differentiation of mitotic progenitor cells into more specialized differentiated cells. Although the cell cycle regulators controlling G1/S in endocycles and mitotic cycles are mostly the same, the mitotic regulators are typically suppressed in endocycling cells (reviewed by De Veylder et al., 2011; DePamphilis, 2016; Edgar and Orr-Weaver, 2001; Edgar et al., 2014; Fox and Duronio, 2013; Orr-Weaver, 2015; Zielke et al., 2013). At least two essential modifications of the mitotic cell cycle must be made in order to switch from a mitotic cell cycle to an endocycle. First, the key events of M phase, sister chromatid separation and cytokinesis, must be suppressed without blocking S phase. This is generally accomplished by downregulating the activity of mitotic cyclin-dependent kinase (M-CDK), which drives G2/M-phase progression, while the activity of the CDK responsible for S-phase entry (S-CDK) is maintained (Fig. 2). In animal cells, M-CDK activity is provided by CDK1 bound to one of its activating subunits, cyclin B or cyclin A, which also confer substrate specificity towards M-phase regulators. By contrast, S-CDK activity is normally provided by CDK2 that is bound and activated by cyclin E or cyclin A. In *Drosophila* salivary glands, Cyclin B, Cyclin A and CDK1 levels are reduced in endocycling cells, whereas they are elevated in mitotic cells (Zielke et al., 2011). In *Oikopleura*, the mitotic cyclins, cyclin A, cyclin B and cyclin B3, and a CDK1 paralog that is essential for oogenic M phase, CDK1d, are all transcriptionally downregulated in endocycling somatic tissue (Campsteijn et al., 2012; Øvrebo et al., 2015). M-CDK downregulation is also sufficient to induce endocycling in many cell types that are not pre-programmed to enter endocycling (Broek et al., 1991; Chen et al., 2016; Hassel et al., 2014; Hayashi, 1996; Hayles et al., 1994; Mihaylov et al., 2002; Sauer et al., 1995; Sigrist and Lehner, 1997; Weigmann et al., 1997), suggesting that M-CDK downregulation may be a widespread means by which to trigger endocycling. However, blocking the factors and pathways that are activated downstream of M-CDK may also be sufficient to induce endoreplication. For example, in the planarian *S. mediterranea*, endoreplication cycles can be induced by blocking chromosome condensation through RNAi-mediated knockdown of condensins (Lai et al., 2017).

A Mitosis



B Endoreplication

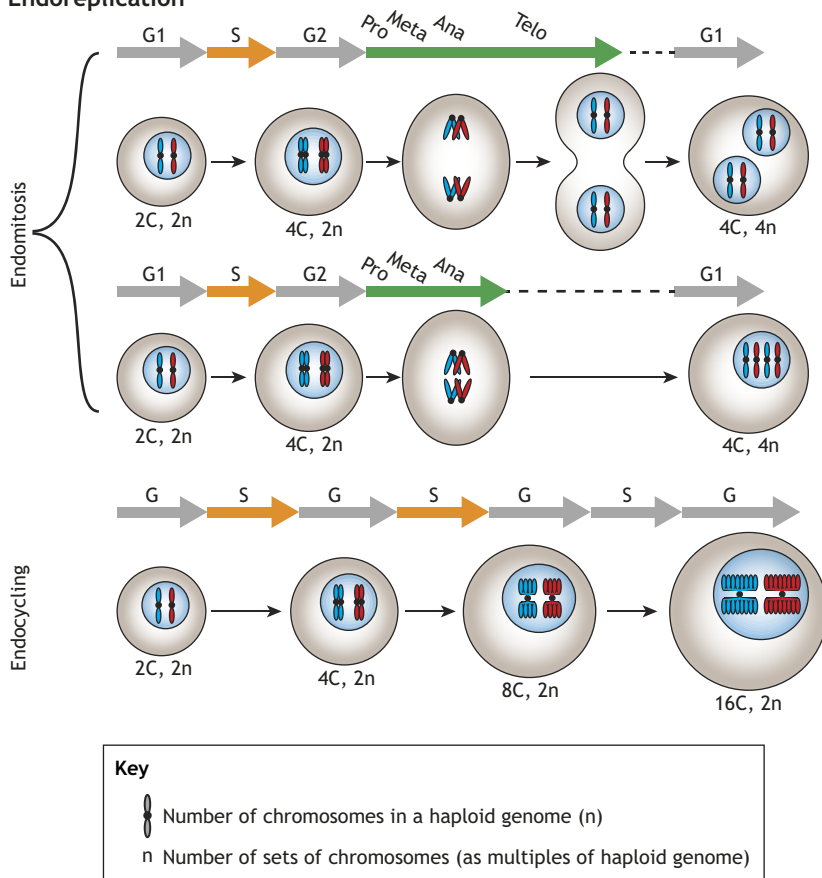


Fig. 1. An overview of alternative cell cycles.

(A,B) While mitosis (A) gives rise to diploid cells, a common path to polyploidy is endoreplication (B), which includes two subgroups: endomitosis and endocycling. Similar to mitotic cells, cells that undergo endomitosis enter the cell cycle, which consists of four canonical phases: G1, S, G2 and M. Endomitosis is characterized by incomplete cytokinesis, thus resulting in a polyploid binucleate cell or a polyploid mononucleate cell. By contrast, endocycling cells lack M phase altogether, resulting in a two-phase cell cycle consisting of alternating G and S phases. Endocycling cells often over- or under-amplify certain genomic regions, resulting in joined polytene chromosomes.

The second requirement for mitosis to endoreplication switching is the re-assembly of the pre-replication complex (pre-RC) during successive G phases. During late M phase, Cdc6 is recruited to origin recognition complexes (ORCs), which are multi-protein complexes assembled at initiation sites for DNA replication (Bell and Stillman, 1992; Riera et al., 2017; Yuan et al., 2017). Cdc6-ORC facilitates the recruitment of two Cdt1-bound Mcm2-7 hexamers on replication origins, forming pre-RCs on licensed origins (Bell and Labib, 2016; Cocker et al., 1996). Origin firing, and the onset of DNA replication, is triggered by S-CDK activity, which prompts the recruitment of multiple additional factors and activates Mcm2-7 DNA helicase activity (Heller et al., 2011; Zegerman and Diffley, 2007). These events ultimately lead to the recruitment of DNA clamps known as proliferating cell nuclear antigen (PCNA) and DNA polymerase, marking the onset of S phase. Cdt1 recruitment and function is repressed by CDK activity (Chen and Bell, 2011; Sugimoto et al., 2004) and pre-RC assembly therefore requires a window of low CDK activity in G phase. Constitutive S-CDK activity can thus block endocycling (Follette

et al., 1998; Remus and Diffley, 2009; Weiss et al., 1998). Although the mechanisms by which CDK activity suppresses pre-RCs have been established in budding yeast, how CDKs might do this in endocycling cells is less clear. Furthermore, and as we detail below, the mechanisms used to block M-CDK and retain S-CDK oscillations in endocycling cells vary widely between cell types and organisms, showing just how versatile the building blocks of the cell cycle are.

Drosophila endocycles

An informative example of the transition from mitosis to endocycling is observed in follicle cells of the *Drosophila* ovary. These cells, which form an epithelium that surrounds the developing *Drosophila* oocyte, proliferate by mitosis up until the 7th stage of oogenesis, at which point the oocyte and polyploid nurse cells that support the oocyte start expressing the Notch ligand Delta (Deng et al., 2001; Lopez-Schier and St Johnston, 2001). Expression of Delta in the germline activates Notch signaling in the surrounding follicle cells. This induces expression of *Hindsight* (*Hnt*), which

Box 2. Polyploidy and cancer

The majority of cancers display aneuploidy, with around 90% of solid tumors and 75% of hematopoietic cancers having abnormal chromosome numbers (Weaver and Cleveland, 2006). Aneuploidy in cancer cells likely originates from previously polyploid cells generated by either cell-cell fusion, which may be induced by viral infection (Duell and Lazebnik, 2007; Duelli et al., 2007), or endoreplication, although some prefer the term 'abortive cell cycle' to distinguish it from developmentally programmed endoreplication (Davoli and de Lange, 2011; Storchova and Pellman, 2004). Tetraploidization has been observed to occur alongside upregulation of Mad2, which downregulates MKlp2, a kinesin required for cytokinesis (Lee et al., 2010). This may enhance the occurrence of abortive cell cycles. Developmentally programmed endoreplication usually occurs alongside irreversible differentiation and termination of proliferation. Normal exceptions include *Drosophila* rectal papillar cells and hepatocytes, which re-enter mitosis (Duncan et al., 2010; Fox et al., 2010). However, genetically unstable cells may be capable of continued proliferation, which may involve reduced stringency of postmitotic cell cycle checkpoints that prevent the proliferation of aneuploid/tetraploid cells (Andreassen et al., 1996; Lanni and Jacks, 1998; Minn et al., 1996). Because centrosomes are also duplicated in S phase, tetraploid cells obtain supernumerary centrosomes before the ensuing M phase. Cells with supernumerary centrosomes are quite prone to chromosome mis-segregation and aneuploidy (Storchova and Pellman, 2004). This hypothesis is supported by the observed enrichment of near-tetraploid aneuploid cells (~4n) in tumors, and aneuploid tumor cells possessing supernumerary centrosomes (Kaneko and Knudson, 2000; Levine et al., 1991; Mitelman, 2005; Reid et al., 1996). Aneuploidy also contributes to rapid cancer cell evolution, because it can give rise to an increasingly heterogeneous population of tumor cells that can undergo selection. Accordingly, aneuploidy in cancer is associated with poor prognosis (Carter et al., 2006; Coward and Harding, 2014; Oltmann et al., 2018; Sheffer et al., 2009; Walther et al., 2008) and increased tolerance to chemotherapy (Lee et al., 2011; Vargas-Rondon et al., 2018). We can draw a parallel with the heterogeneity and increased fitness created by mitosis of tetraploid hepatocytes, and speculate that aneuploidy in cancer cells turns this otherwise beneficial mechanism to its selfish advantage.

encodes a transcription factor that represses expression of the essential CDK1 activator String/Cdc25 (Fig. 3). The loss of *String/Cdc25* expression thus precludes M-phase entry and causes a prolonged arrest in G2 (Schaeffer et al., 2004; Shcherbata et al., 2004; Sun and Deng, 2007). In addition to repressing *String/Cdc25* expression, Hnt represses expression of *Cut*, another transcription factor gene, and this allows accumulation of Fzr/Cdh1, an activating subunit of the anaphase promoting complex/cyclosome (APC/C) (Narbonne-Reveau et al., 2008; Sun and Deng, 2007). In contrast to the other activating subunit of APC/C, Fzy/Cdc20, Fzr/Cdh1 does not require activation by M-CDK activity and promotes degradation of the mitotic cyclins (A, B and B3), thus maintaining a window of low CDK activity during G1. Fzr/Cdh1 also targets Geminin, a protein that prevents DNA re-replication by binding Cdt1, for proteasomal degradation. The activity of Fzr/Cdh1 is thus sufficient to allow re-assembly of pre-RCs in G1, and guides G2-arrested cells back into a G1-like state ready for DNA replication (Sun and Deng, 2007). The follicle cells can therefore re-enter S phase once S-CDK activity reaches sufficient levels to fire pre-RCs. Similarly, Delta-Notch signaling promotes the mitosis-to-endocycle switch during the differentiation of enterocytes in the adult *Drosophila* midgut and of larval glial cells (Von Stetina et al., 2018; Xiang et al., 2017). However, Delta-Notch signaling has exactly the opposite effect in follicle cells in a distantly related insect, the flour beetle *Tribolium* (Bäumer et al., 2012), suggesting that upstream regulatory inputs into the mitotic-endocycle switch are not evolutionarily conserved.

The mechanisms that promote downregulation of mitotic cyclins can also differ across tissues. For example, although mitotic cyclins are downregulated post-transcriptionally in *Drosophila* follicle cells, they are transcriptionally silenced in endocycling *Drosophila* salivary glands (Maqbool et al., 2010; Zielke et al., 2008).

Transcriptional regulation is also important for continued progression through endocycling. *Drosophila* has a single activating member of the E2F family of transcription factors, E2F1, that binds DNA as a heterodimer with 'dimerization partner' (DP). *Drosophila* also has a repressor E2F, E2F2, that represses transcription of E2F1-DP targets as part of the Myb-MuvB complex. E2F1-DP regulates several M-phase genes and genes required for DNA replication, and dampened E2F1 activity is required for endocycles in fat bodies and salivary glands (Maqbool et al., 2010). Disturbing the balance of E2F1-DP/Myb-MuvB activity, either through gene silencing or overexpression, disrupts endocycles and reduces the ploidy of these tissues (Maqbool et al., 2010). This observation suggests that reduced E2F1-DP activity is required to dampen the expression of M-phase genes, whereas minimal activity is needed to maintain transcription of *cyclin E* and other S-phase regulators. Similar to cyclins, *Drosophila* E2F1 displays oscillating protein expression, such that its accumulation peaks by the end of G1 phase, followed by proteasomal degradation during S phase. This degradation of E2F1 is directly linked to DNA replication: chromatin-bound PCNA binds E2F1 through a 'PCNA interacting protein (PIP) motif', which mediates E2F1 proteolysis through the CRL4^{cdt2} ubiquitin ligase (Shibutani et al., 2008). Although mammalian E2F factors (E2F1-E2F3) are not known to be regulated by PIP-motif targeted degradation during S phase, human E2F1-E2F3 also display cyclic activity both through cyclic competition with the atypical E2Fs, E2F7/8, and via cyclic degradation by SCF^{Skp2} and APC/C^{cdc20} in S/G2 and M phases, respectively (de Bruin et al., 2003; Maiti et al., 2005; Marti et al., 1999; Peart et al., 2010; Wong et al., 2011).

Mammalian endocycles

One of the best studied examples of endocycling in mammals is in murine trophoblast giant cells (TGCs). These cells form the outermost layer of the placental extraembryonic compartment and produce a number of pregnancy-specific cytokines and hormones. TGCs undergo a mitosis-endocycle switch during embryogenesis, and failure to endocycle compromises normal TGC development and affects fetal viability (Ouseph et al., 2012). They are believed to completely replicate their genome, in contrast to the polytene chromosomes of *Drosophila* salivary glands (Sher et al., 2013), although some genomic regions containing key placental genes are amplified (Hannibal and Baker, 2016). Diploid TGC progenitors and trophoblast stem cells (TSCs) can be cultured *in vitro*, where they can be experimentally induced to switch to an endocycle. Studies of such cultured cells have revealed that, similar to *Drosophila* endocycling cells, TGCs sustain downregulation of M-CDK activity as they switch to endocycling (Hochegger et al., 2007; Ullah et al., 2008).

The selective inhibition of CDK1 activity is sufficient for TSCs to differentiate into TGCs (Ogawa et al., 2016). TSCs remain undifferentiated through epigenetic modification by TET1 and TET2, which promote demethylation of chromatin (Chrysanthou et al., 2018; Tahiliani et al., 2009). As TSCs differentiate, TET1 and TET2 are downregulated, causing increased expression of the cyclin-dependent kinase inhibitor (CKI) p21^{Cip1} and enhanced cyclin B degradation (Chrysanthou et al., 2018). The induction of endocycling by withdrawal of the fibroblast growth factor FGF4

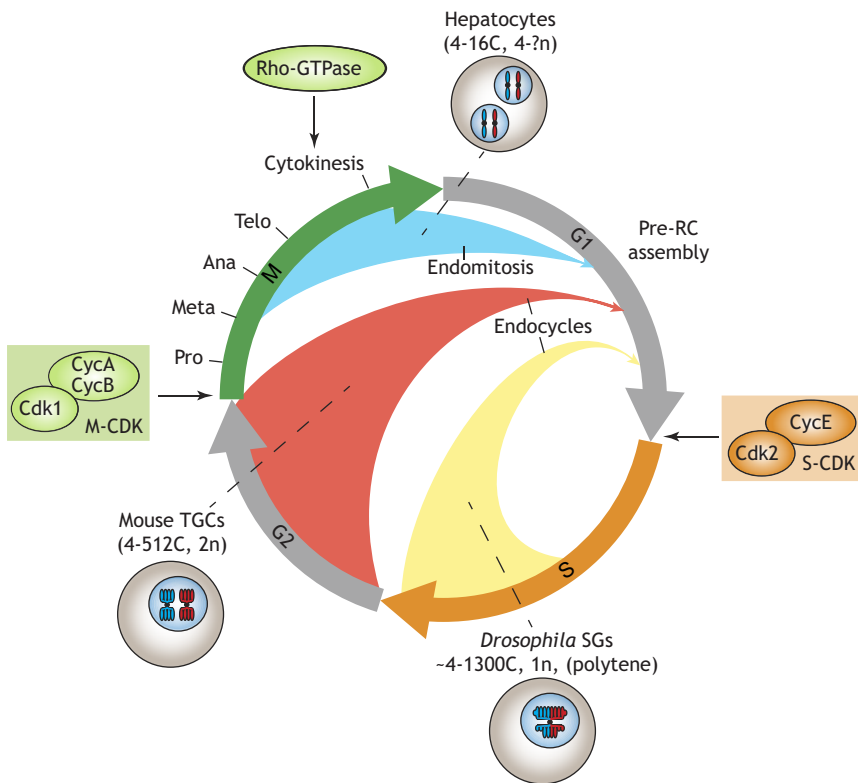


Fig. 2. Alternative cell cycles found in development and regeneration. The mitotic cell cycle is composed of four phases: G1, S, G2 and M. The G1/S and G2/M cell cycle transitions are controlled by S-CDK and M-CDK activities, respectively. Endopolyploidy arises from altered cell cycles in which different cell cycle phases are truncated or bypassed entirely (blue, red and yellow arrows). As examples, mouse hepatocytes skip only cytokinesis in a cell cycle variant known as endomitosis (blue arrow), mouse trophoblast giant cells (TGCs) enter G1 following G2 (red arrow) and *Drosophila* salivary gland cells (SGs) re-enter a G1-like phase before fully completing DNA replication in S phase (yellow arrow). Endocycles and endomitoses are frequently regulated through downregulation of M-CDK and cytokinesis, respectively.

(Tanaka et al., 1998) also promotes the accumulation of p21^{Cip1} and another CKI, p57^{Kip2}, which consequently inhibits CDK1 and suppresses mitosis (Ullah et al., 2008) (Fig. 4). In this context, upregulation of p57^{Kip2} and p21^{Cip1} appears to be controlled by checkpoint kinase-1 (Chk1), a component of the DNA damage checkpoint (Ullah et al., 2011). In mitotically proliferating TGCs, p57^{Kip2} and p21^{Cip1} are targeted for proteasomal degradation upon phosphorylation by Chk1. Upon FGF4 deprivation, however, Chk1 is silenced, allowing accumulation of p57^{Kip2} and p21^{Cip1}. In this context, p57^{Kip2} translocates to the nucleus where it appears to be the main mediator of the endocycle switch, as evidenced by the fact that p21^{Cip1}-deficient TGCs endocycle normally, whereas p57^{Kip2}-deficient trophoblasts are hyper-proliferative and cause hyperplasia and placentalomegaly (Ullah et al., 2008). In contrast to p57^{Kip2}, p21^{Cip1} localizes to the cytoplasm, where it protects TGCs from DNA damage-induced apoptosis (de Renty et al., 2014).

Changes in G1/S transcriptional control also play an important role in the TGC endocycle switching. This control involves the E2F family of transcription factors, which in mammals includes the activator E2Fs (E2F1-E2F3), the repressor E2Fs (E2F4-E2F6), and two atypical repressor E2Fs (E2F7 and E2F8) (Chen et al., 2009; van den Heuvel and Dyson, 2008). Activity of the E2Fs is also modulated by their activating dimerization partners (Dp1, Dp2 and Dp4) and repressed by retinoblastoma-like pocket proteins (Rb, p107 and p130). Rb-deficient TGCs display excess mitotic proliferation, caused by elevated E2F3a activity (Chong et al., 2009; Wenzel et al., 2007), whereas deletion of all three activator E2Fs results in increased TGC ploidy (Chen et al., 2012). Conversely, deletion of the two atypical repressor E2Fs, E2F7 and E2F8, leads to reduced TGC ploidy, which favors mitotic proliferation. Interestingly, E2F8 is also indispensable for the polyploidization of endometrial stromal cells, which is associated with ERK- and STAT3-dependent E2F8 expression and suppressed CDK1 activity (Qi et al., 2015). These observations suggest that

E2F1-E2F3 activity promotes mitosis and needs to be suppressed for correct switching to endocycling (Chen et al., 2012). This idea is supported by the fact that knockouts of E2F1 or E2F3 are sufficient to rescue endocycle defects caused by an E2F7/E2F8 double deletion (Chen et al., 2012; Ouseph et al., 2012). Considering this, it is somewhat puzzling that cyclin E1, a transcriptional target of E2F1-E2F3 (Parisi et al., 2003), is essential for endocycling in TGCs. This paradox might be explained by alternative regulation of cyclin E1 expression, for example by Myc, in parallel with E2F activity (Santoni-Rugiu et al., 2000). Overall, these observations imply that mammalian activator E2Fs restrict endocycling and underscore the notion that the balance between activator and repressor E2Fs contributes to the switch from mitotic cycling to endocycling.

The upstream regulation of endoreplication and ploidy

Endocycling rates can often be dictated by environmental factors, such as nutrition or stress. For example, sunlight can affect whether plant leaf cells undergo mitotic cycles or endocycles (Berckmans et al., 2011; Gendreau et al., 1998). However, superimposed upon such environmental regulation, developmentally programmed regulation often dictates the final ploidy achieved by endocycling (Audibert et al., 2005; Hammond and Laird, 1985; Unhavaithaya and Orr-Weaver, 2012). Thus, under optimal nutritional conditions, the larval salivary gland cells of *Drosophila* typically reach a ploidy of ~1300C, while its fat body cells achieve ploidies of ~256C and adult midgut enterocytes gain a maximum ploidy of 32C (Butterworth and Rasch, 1986; Hammond and Laird, 1985). In these cases, final ploidy is likely to be controlled by both a developmental time window and gap (G) phase durations. For example, the time window that supports endocycles starts when CDK1 activity is suppressed and lasts for as long as essential cell cycle regulators such as E2F1 and Cyclin E are expressed. In addition, the number of endocycles that occur within a particular

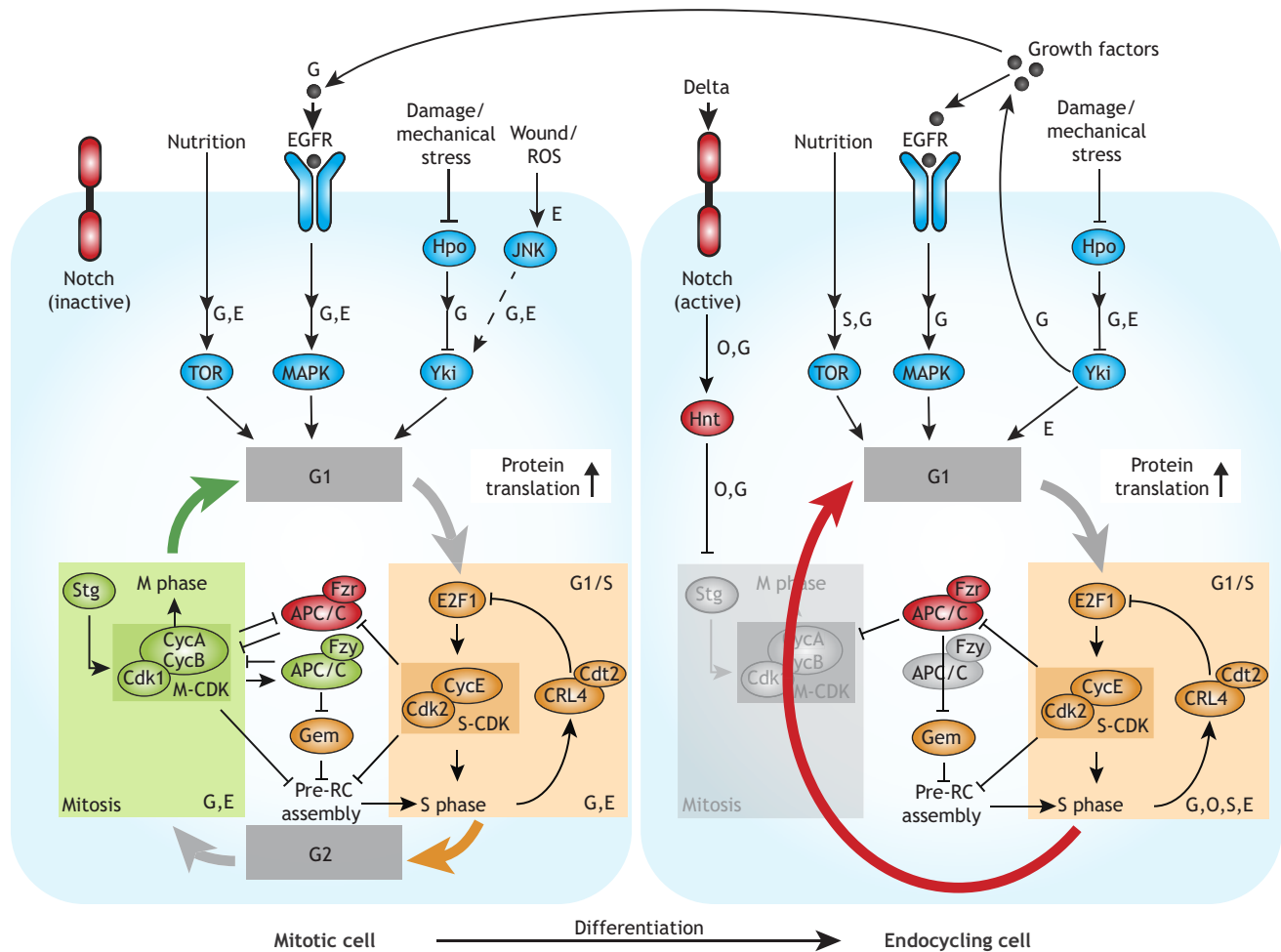


Fig. 3. Mitotic-endocycle transitions in *Drosophila*. Cell growth in *Drosophila* is controlled by multiple pathways, including the PI3K/TOR, EGFR/MAPK, JAK/STAT, JNK and Hippo (Hpo)/Yki pathways; pathways shown to operate in ovarian follicle cells (O), adult midgut (G), salivary gland (S) and epidermis (E) are indicated. Cellular growth rates affect levels of E2F1 protein, which controls G1/S in a rate-limiting manner through transcriptional activation of Cyclin E (CycE), which binds to and activates CDK2, forming active S-CDK complexes. S phase triggers proteasomal degradation of E2F1 through activation of CRL4^{cdt2}. The G2/M transition is regulated by CycA- and CycB-dependent CDK1 kinase activity (M-CDK), which requires activation by String (Stg); a Cdc25-type phosphatase). M-CDK kinase activity activates the APC/C subunit Fizzy (Fzy). The E3 ligase APC/C^{Fzy} targets Geminin (Gem), CycA and CycB for proteasomal degradation; the subsequent depletion of Geminin and M-CDK activity relieves inhibition of Cdt1 and creates a window of low CDK activity (not shown), respectively, which allows re-assembly of the pre-replication complex (pre-RC). In the adult midgut (G) and follicle cells of the ovary (O), mitosis-endocycle transitions are triggered through expression of the Notch ligand Delta in adjacent cells. In follicle cells, active Notch induces *Hindsight* (*Hnt*), which represses *Stg* expression and thereby blocks M-CDK activity. Upregulation of *Fizzy-related* (*Fzr*), another activating subunit of APC/C that does not require activation by M-CDK, ensures low M-CDK activity and mediates destruction of Geminin, which in turn allows pre-RC assembly while bypassing M phase. Increased endocycling can also be induced by mechanical stress via the Hippo pathway and JNK. The Hippo pathway stimulates increased ploidy non-cell-autonomously in enterocytes of the adult midgut (G) through expression and secretion of cytokines and growth factors, which activate the EGFR/Ras/MAPK and JAK/STAT pathways, increasing cell growth rates and decreasing the length of G phase. In the adult epidermis (E), Yorkie (Yki) is required for polyploidization cell-autonomously upon wound closure.

developmental time window is likely determined by the length of each cycle, which is mostly dictated by G-phase length. Duration of G phase, in turn, is decided by the overall growth rate of the cell, which is decided by environmental factors such as nutrition, growth signals and stress. For example, it has been shown that in *Drosophila* salivary glands, increased growth rate is followed by an increased rate of protein synthesis, followed by increased accumulation of cell cycle regulators, including E2F1, which leads to faster transition to S phase and thus less time spent in G phase (Zielke et al., 2011). As the length of endocycle G phases appears to be controlled by the same gene products that control G1/S-phase progression in mitotic cells [namely CDK2/cyclin complexes, CDK inhibitors (CKIs) and E2F/Rb, etc.], it should come as no surprise

that mitotic cycles and endocycles share the same upstream regulators of G1/S-phase progression. A number of such upstream regulators have been identified. The polyploidization of megakaryocytes, for example, is induced by thrombopoietin (McDonald, 1992), a glycoprotein hormone secreted from liver and kidney. Thrombopoietin regulates G1/S phase, by controlling cyclin E expression, in endomitotic megakaryocytes via the STAT pathway (Eliades et al., 2010; Kaushansky, 2016). Other pathways and factors affecting megakaryocyte polyploidization include PI3K/Akt, MAPK/ERK and Myc (Chanprasert et al., 2006). Earlier studies also demonstrated that nutrient availability and protein synthesis rates, which depend on Myc and TOR activity, are tightly linked to endocycle speed and final ploidies (Britton and Edgar,

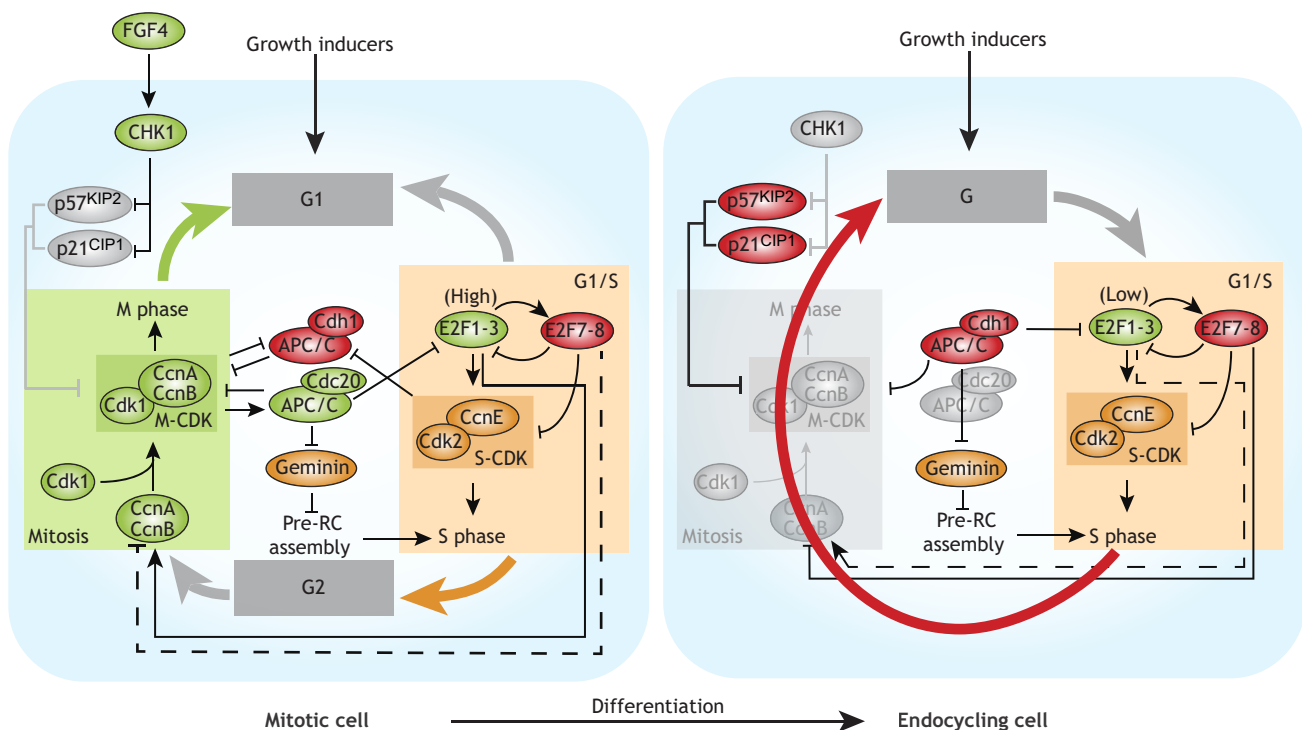


Fig. 4. Mitotic-endocycle transitions in trophoblast giant cells. Cellular growth rates affect activation of E2F1-3, which control G1/S through transcriptional activation of S-CDK activity. E2F1-3 also activate expression of E2F7-8, which in turn repress expression of E2F1 and its targets, thus forming a negative-feedback loop. E2F1-3 are also required for the expression of cyclin A and cyclin B (CcnA and CcnB), which activate CDK1 (M-CDK) and are required for M-phase entry. M-CDK kinase activity activates the APC/C subunit Cdc20. The E3 ligase APC/C^{Cdc20} targets geminin, CcnA and CcnB for proteasomal degradation. Depletion of geminin and M-CDK activity relieves inhibition of Cdt1 and creates a window of low CDK activity, respectively, which allows re-assembly of the pre-replication complex (pre-RC). *In vitro*, trophoblast stem cells are induced to enter endocycles upon FGF4 deprivation, which reduces CHK1 activity and relieves degradation of p57^{Kip2} and p21^{Cip1}. Accumulation of p57^{Kip2} and p21^{Cip1} block M-CDK activity, which establish an M-phase bypass and onset of endocycles in TGCs. Further polyploidization is also affected by E2F7-8-dependent downregulation of E2F1-3.

1998; Britton et al., 2002; Demontis and Perrimon, 2009; Grewal et al., 2005; Pierce et al., 2004; Saucedo et al., 2003). In *Drosophila* larval salivary glands and adult midgut enterocytes, endocycle rates appear to be controlled, downstream of the TOR and EGFR pathways, respectively, by the post-transcriptional expression of the single *Drosophila* activator E2F: E2F1 (Xiang et al., 2017; Zielke et al., 2011). In TGCs, however, endocycles are promoted by elevated E2F8 expression (Qi et al., 2015), which is induced by progesterone through the EGFR/ERK/STAT3 signaling pathway. Paradoxically, the activator E2Fs are dispensable for TGC and hepatocyte endoreplication in triple knockout (E2F1-3) mice (Chen et al., 2012), where it remains unclear how S-CDK and DNA replication factor gene expression is sustained.

Although metabolism and growth may affect endocycle speed in many contexts, exit from the endocycle in some cases may be controlled separately by developmental induction of CKIs or transcriptional downregulation of S-CDK activity. This latter mechanism might be achieved through a switch from activating to repressive E2F activity, followed by a 'lock down' of G1/S-phase regulators by chromatin remodeling complexes (Buttitta and Edgar, 2007). In this way, upstream regulators may define a developmental time-window during which growth-dependent endocycling is allowed. For example, *Drosophila* ovarian follicle cells exit endocycles in response to developmentally controlled downregulation of Notch receptor activity, which coincides with decreasing levels of the Notch ligand Delta being expressed in oocytes. Loss of Notch activity then causes increased activity of Tramtrack, a transcriptional repressor downstream of the ecdysone receptor (EcR) signaling pathway, the

activity of which is required for termination of endocycling (Sun et al., 2008).

Polyploidization via cell fusion

Sometimes polyploidy does not arise directly from cell cycle modifications, but instead occurs following the fusion of two neighboring cells to produce a cell with increased ploidy. A well-known example of cell-cell fusion is the fusion of two haploid germ cells, which gives rise to a diploid zygote. However, there are also examples of diploid somatic cells that fuse together to form polyploid multinuclear cells. These include vertebrate and *Drosophila* myoblasts and mammalian osteoclasts (Chen and Olson, 2004; Kim et al., 2015a; Xing et al., 2012).

Our best understanding of cell-cell fusion derives from studies of myoblasts in *Drosophila*, zebrafish and mice (Kim et al., 2015a). In this context, cell-cell fusion requires cell-cell adhesion followed by enhancement of cell membrane proximity and destabilization of lipid bilayers. In *Drosophila*, cell-cell adhesion and recognition are mediated by immunoglobulin domain-containing cell-adhesion molecules (CAMs): the formation of new muscle fibers is seeded by founder cells expressing the CAMs Dumbfounded/Kin-of-IrreC (Duf/Kirre) and Roughest (Rst), which are attracted by fusion-competent myoblasts (FCMs) expressing a CAM named Sticks and stones (Sns) (Bour et al., 2000; Ruiz-Gómez et al., 2000; Strunkelberg et al., 2001). In zebrafish myoblasts, cell adhesion is established by the Duf/Kirre homolog Kirrel (Srinivas et al., 2007), but whether two different cell types are also required in vertebrates is not known. In *Drosophila*, membrane proximity is

then enhanced by F-actin-enriched podosome-like structures formed in FCMs, which protrude towards the founder cell membrane. This protrusion is propelled by actin polymerization regulated by the Arp2/3 complex (Berger et al., 2008; Massarwa et al., 2007; Richardson et al., 2007). Membrane proximity is further enhanced by a mechanosensory response in founder cells, creating protrusion resistance through Myosin II-induced cortical tension. The accumulation of MyoII activity ultimately promotes the formation of a fusion pore, joining the two fusing cells. In mice, exposure of phosphatidylserines to the cell surface has been proposed to be involved in membrane destabilization during fusion pore formation (Jeong and Conboy, 2011; Kim et al., 2015b; van den Eijnde et al., 2001). Similar to several endocycling cell types in *Drosophila*, Delta-Notch signaling is involved in the differentiation of FCMs, whereas Ras controls the differentiation of founder cells (Artero et al., 2003).

Stress- and injury-induced polyploidy

Injury, as well as cellular stress, can cause loss of cells and hence loss of tissue integrity. In such cases, cell mass needs to be restored in order to maintain tissue function and homeostasis. In most regenerative tissues, lost cells are replaced by cell division of nearby progenitor cells or resident stem cells. However, recently reported examples show that some tissues also use endocycles and/or cell-cell fusion to compensate for losses of tissue mass. Together, these findings illuminate a new aspect of wound healing that has the potential to open up novel strategies for regenerative medicine. Below, we discuss how the control of ploidy contributes to tissue regeneration in the *Drosophila* gut and epidermis, and in the vertebrate heart and liver.

Polyploidy in the regenerating *Drosophila* gut

The *Drosophila* intestinal epithelium is composed predominantly of a monolayer of polyploid absorptive cells called enterocytes (ECs). These terminally differentiated cells arise from progenitor cells, known as enteroblasts (EBs) (Ohlstein and Spradling, 2007), that undergo up to four endocycles, giving rise to polyploid ECs with a final ploidy of 8–32C (Edgar et al., 2014). Upon EC loss and stress, which can be caused by cytotoxic exposure or enteric infection, tissue homeostasis is maintained by a pool of resident intestinal stem cells (ISCs) that divide to produce committed post-mitotic EBs. Specifically, damaged ECs stimulate the proliferation and differentiation of neighboring ISCs via the secretion of cytokines and EGFR ligands, which are regulated by signaling pathways such as the JNK and Hippo (Hpo) pathways (Huang et al., 2005; Shaw et al., 2010; Staley and Irvine, 2010; Zhou et al., 2017).

The Hpo pathway is an evolutionarily conserved pathway that senses structural integrity and changes in cell adhesion to regulate cell proliferation and survival (Dupont, 2016) (Fig. 3). The activity of the protein kinase Hpo is disrupted by tissue damage through a mechanism that involves signals derived from organization of the F-actin cytoskeleton, which modulates the activity of the Hpo pathway effector Yorkie (Yki) according to cell adhesion, cell density and actin filament tension (Rauskolb et al., 2014; Varelas et al., 2010; Zhao et al., 2012). In short, loss of Hpo activity allows nuclear localization of Yki and transcriptional activation of numerous genes involved in cell growth and other processes (Huang et al., 2005). In the fly gut, Yki also stimulates the transcription of secreted cytokines and growth factors, which stimulate Janus Kinase (JAK) and EGFR activity, respectively, promoting faster growth and endocycling in differentiating EBs (Furriols and Bray, 2001; Houtz et al., 2017; Ohlstein and Spradling, 2007; Ren et al., 2010; Shaw

et al., 2010). Similarly, damage and ROS activate ISC proliferation through JNK activation in ECs, which stimulates the secretion of cytokines upon injury (Jiang et al., 2009; Santabarbara-Ruiz et al., 2015).

EBs endocycle in response to Notch signaling, although how M-CDK activity is suppressed by Notch remains obscure. In addition, although ECs from healthy guts are known to require TOR activity for scheduled endocycling (Xiang et al., 2017), EGFR signaling has been demonstrated to control damage-induced endocycling independently of TOR signaling, via post-transcriptional upregulation of E2F1 (Xiang et al., 2017). Thus, upon damage, EGFR signaling promotes compensatory polyploidization of ECs and, surprisingly, Insulin/PI3K/TOR signaling becomes dispensable. This example demonstrates a conditional ‘switching’ of pathways controlling endocycles, with one input pathway controlling endocycling during normal growth and another during stress-induced regeneration. E2F1 has also been demonstrated to be a rate-limiting G1/S-phase regulator in numerous *Drosophila* tissues, including the embryonic epidermis, larval salivary glands, fat body, gut, imaginal discs (epidermal progenitors) and the adult gut (Britton and Edgar, 1998; Duronio and O’Farrell, 1995; Follette et al., 1998; Maqbool et al., 2010; Xiang et al., 2017; Zielke et al., 2011). E2F1 therefore has a key role as a growth-sensing cell cycle regulator in *Drosophila*.

Wound healing and polyploidy in the *Drosophila* epidermis

There are many adult *Drosophila* tissues that lack stem cells and that therefore must accomplish wound healing via other mechanisms. The epidermis of adult *Drosophila*, located beneath the cuticle, is one such tissue. This tissue normally consists of a continuous layer of diploid ectodermal epithelial cells. However, if this epithelium is damaged by a puncture wound, it heals by complete wound closure in the absence of mitoses (Losick et al., 2016) (Fig. 5). As is the case for the regenerating *Drosophila* gut, this wound-healing process involves JNK and the Hpo pathway effector Yki. Interestingly, JNK has been reported to cross-regulate the Hpo pathway in wing discs (Enomoto et al., 2015).

Shortly after wounding, Yki and JNK activities increase in cells surrounding the wound site. Epithelial cells closest to the site of injury slowly migrate towards the center of the wound, and multiple cells fuse together to form a syncytium. This effect is dependent on Rac GTPase activity (Losick et al., 2013), which is also known to control cell fusion, myoblast migration and epithelial wound closure in the *Drosophila* embryo (Fernandes et al., 2005; Verboon and Parkhurst, 2015). As in the adult, wound closure in the *Drosophila* larval epithelium deploys cell-cell fusion around a wound site. In this context, cell-cell fusion is promoted by JNK activity, which is elevated around the wound site, while cell fusion is suppressed by JAK/STAT activity distal from the wound site (Lee et al., 2017). How JNK promotes cell-cell fusion is not well understood, but may involve JNK-dependent upregulation of integrins (Lee et al., 2017; Wang et al., 2015). In the adult fly epithelium, the downstream targets of JNK, Jra/Kay (Jun/Fos), modulate the activity of Yki by dampening its effect on polyploidization (Losick et al., 2016). However, JNK is also known to increase Yki activity in imaginal discs through inhibition of Warts, or through direct activation (Bunker et al., 2015; Sun and Irvine, 2013). It thus seems that JNK may affect Yki activity in several different tissue- or context-specific ways. In the adult epithelium, cells more distal to the wound site compensate for lost cells by endocycling and increasing cell growth, while leading-edge cells migrate toward the center of the wound. This endocycling response is dependent on Yki, the nuclear translocation and activation

A *Drosophila* adult epithelium

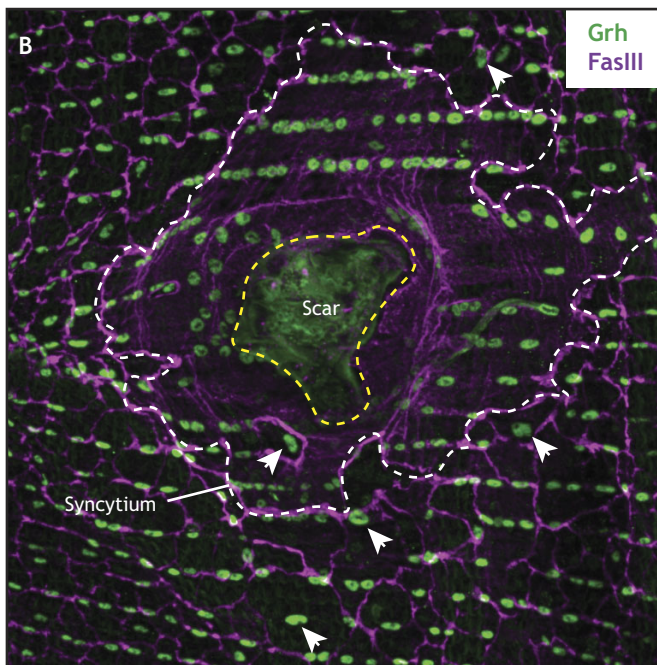
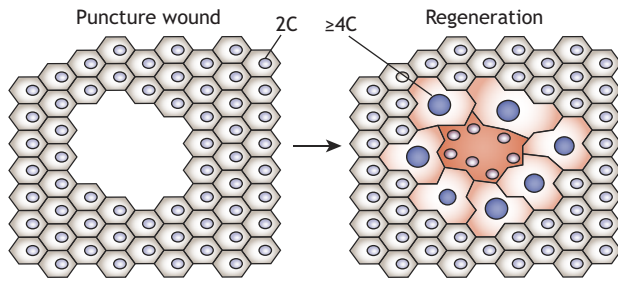


Fig. 5. Wound-induced polyploidization in the adult *Drosophila* epithelium. (A) The adult *Drosophila* epithelium is composed of post-mitotic diploid cells. Upon epithelial puncture wounds, diploid epithelial cells are lost and the open wound is closed by surrounding cells that fuse together to form a syncytium. Cells surrounding the central syncytium undergo endocycles and promote compensatory growth. (B) Immunofluorescence image of regenerating *Drosophila* adult epithelium. Epidermal nuclei and cell-cell septate junctions, marked by Grainy-head (Grh, in green) and Fas3 (in magenta), respectively, are shown. The boundaries of the scar and syncytium are outlined in yellow and white dashed lines, respectively. Examples of large polyploid nuclei are indicated by arrowheads. Image courtesy of Vicki Losick (see Losick et al., 2013 for details).

of which are likely induced by disruption of cell-cell contacts, and/or on stretching of the epithelium (Aragona et al., 2013; Gaspar and Tapon, 2014). Yki has previously been shown to upregulate Cyclin E in wing and eye imaginal discs, and both Yki and Cyclin E are required for compensatory proliferation in these contexts (Meserve and Duronio, 2015; Shu and Deng, 2017; Udan et al., 2003). Cyclin E may therefore be an essential target of Yki during compensatory endocycling and cell growth in *Drosophila*. Of note, Yki is also required for wound closure in the post-mitotic polyploid larval epithelium, which also occurs independently of mitotic proliferation. In this context, however, Yki stimulates wound closure through polymerization of actin filament cables and does not induce further polyploidization or cell fusion, again suggesting that the mode of contribution of Yki to wound healing is somewhat tissue specific (Tsai et al., 2017).

Polyploidization and heart regeneration

The contribution of polyploidization to wound healing has also been studied in the mammalian heart. The mammalian heart is known to contain polyploid cells: by adulthood, up to 70% of human and 85% of rodent cardiac myocytes are polyploid (Mollova et al., 2013; Soonpaa et al., 1996). Murine cardiomyocytes stop proliferation during postnatal development, when M-CDK activity is blocked through downregulation of its activator, Cdc25 (Kang et al., 1997; Soonpaa et al., 1996; Tane et al., 2014b). Both M- and S-CDK activity are also reduced by upregulation of CKIs (p21^{Cip1}, p27^{Kip1}) (Poolman et al., 1998; Tane et al., 2014a). However, a wave of S- and M-CDK activity triggers one additional S phase at postnatal day 5, followed by an endomitotic event that results in binucleation of 80–90% of cardiomyocytes during the two first weeks after birth (Soonpaa et al., 1996). This process is dependent on the orphan cyclin, cyclin G1 (Liu et al., 2010). Like the murine heart, cells in the human heart become polyploid after birth, although in this case the cardiomyocytes remain mononucleate (Mollova et al., 2013).

Neonatal mammalian and adult zebrafish hearts display great regenerative capacity, through compensatory mitotic proliferation of pre-excising cardiomyocytes (Jopling et al., 2010; Kikuchi et al., 2010; Porrello et al., 2011). By contrast, postnatal mammalian hearts regenerate poorly and show limited cardiomyocyte proliferation. In this context, compensation for lost cells or cardiac stress, such as that caused by myocardial infarction, is therefore supported by further polyploidization and post-mitotic/hypertrophic growth (Ebert and Pfister, 1977; Senyo et al., 2013; Soonpaa and Field, 1997). Little is known about the signals that stimulate polyploidization in mammalian hearts, but a recent study in zebrafish has demonstrated that mechanical stretching of epicardial cells (the mesothelial cells that cover the heart) grown in an elastic growth chamber is sufficient to induce endocycling and endomitosis through tension (Cao et al., 2017). This artificial tension mimics the physical stretching suffered by epicardial cells, as they migrate towards a wound site, while retaining existing cell-cell adhesions, to seal the wound (Cao et al., 2017). This study also demonstrated that polyploid cells envelop the damaged heart more efficiently than diploid cells. Interestingly, in the zebrafish model, these polyploid cells are formed transiently upon heart injury, and apoptose once regeneration is complete, leaving behind diploid cells. Although polyploid cells facilitate regeneration of the zebrafish epicardium, it has recently been reported that ectopic polyploidization of the myocardium limits the regenerative capacity of zebrafish hearts (Gonzalez-Rosa et al., 2018). This observation suggests that polyploidization in general presents a physiological challenge to regeneration in heart tissues, thus mobilization of mitotic cell division, through cell reprogramming, may be promising as a therapeutic strategy (Srivastava and DeWitt, 2016; Tzahor and Poss, 2017).

Polyploidization during liver regeneration

The liver is also known for its remarkable regenerative capacity; this is not surprising, as it must endure chemotoxic stress from ingestion of toxins from various food sources. Liver polyploidization occurs postnatally and is responsive to both developmental and stress-linked inputs. As such, the postnatal liver becomes increasingly enriched in polyploid cells. Indeed, the human liver is composed of more than 20% polyploid cells at adulthood, whereas rodent livers are more than 70% polyploid (Wang et al., 2017), as a result of endoreplication. Developing hepatocytes undergo endomitosis, generating multi-nucleate cells, which can later divide to produce cells with a 4C or 8C DNA content (Guidotti et al., 2003; Margall-Ducos et al., 2007). Interestingly, blocking mitosis through

conditional knockout of CDK1 in mouse liver does not impair regeneration upon 70% partial hepatectomy, indicating that regeneration can be maintained by hypertrophic growth of polyploid cells (Diril et al., 2012). Upon 30% partial hepatectomy, wild-type mouse livers regenerate primarily through compensatory growth by polyploid cells. Upon 70% hepatectomy, however, polyploid hepatocytes respond with hypertrophic growth, followed by cell division of binucleate hepatocytes to increase cell numbers (Miyaoka et al., 2012).

The molecular pathways and factors controlling liver polyploidization have been investigated. These studies have shown, for example, that oxidative stress, which is caused by pathological stress such as in non-alcoholic fatty liver disease, limits M-CDK activity and leads to the formation of highly polyploid ($\geq 8C$) mono-nucleated hepatocytes (Gentric et al., 2015). However, although endoreplication facilitates liver regeneration, this example also illustrates that excessive endoreplication can be associated with pathological conditions (Gentric and Desdouets, 2015). As with *Drosophila* endocycling cells, mouse hepatocytes need to downregulate mitotic regulators in order to bypass full mitosis, while maintaining expression of cyclin E1 to sustain DNA replication (Chen et al., 2012; Nevzorova et al., 2009). The literature indicates that E2F1-E2F3 promote expression of multiple mitotic genes in hepatocytes, including cyclin A and cyclin B, whereas E2F7 and E2F8 promote endoreplication by repressing expression of cyclin A1 and cyclin A2 (Chen et al., 2012). Thus, the double knockout of E2F7 and E2F8 causes increased cyclin A1 expression and a failure to endoreplicate, while endoreplication can be restored in E2F7- and E2F8-deficient hepatocytes when combined with the knockout of cyclin A1 and cyclin A2 or their transcriptional activator, E2F1 (Chen et al., 2012; Kim et al., 2016; Pandit et al., 2012). This is consistent with the reduced ploidy observed in E2F7/E2F8 double-knockout TGCs.

As with the *Drosophila* epidermis, the Hpo pathway appears to play a key role in liver polyploidization. The mammalian Yki homolog, yes-associated protein (YAP), is required for hepatocyte polyploidization (Zhang et al., 2017). YAP has been found to elevate the activity of Akt, which activates the acetyltransferase p300 by phosphorylation (Zhang et al., 2017) (Fig. 6). p300 then acetylates the F-box protein Skp2, which serves as a substrate recognition component of the Skp1, cullin 1, F-Box (SCF) E3 ligase complex. This acetylation displaces Skp2 from the nucleus to the cytoplasm, thus preventing the Skp2-dependent targeting of nuclear p27 for ubiquitylation and proteasomal degradation, and thereby promoting nuclear accumulation of p27. The net result of this cascade is that YAP elevates p27 levels to suppress M-CDK activity and block cell division (Zhang et al., 2017). How p27 allows enough CycE-CDK2 activity to promote S-phase entry, while at the same time preventing CycB/CDK1 activity and M-phase entry is not yet fully understood. One possible mechanism is that p27 limits the overall CDK activity to a level below the required M-phase threshold, while allowing a level sufficient for S-phase entry, consistent with a mechanism proposed earlier (Edgar et al., 2014; Stern and Nurse, 1996).

In murine livers, E2F8 activity has also been shown to be required for hepatocyte binucleation, where it silences expression of cytokinetic regulators, such as *Racgap*, *Ect2* and *Mklp1* (Pandit et al., 2012). Recently, expression of the micro-RNA miR-122 was shown to be required for hepatocyte bi-nucleation, through silencing of a number of pro-cytokinetic effectors including *Cux1* (Hsu et al., 2016), which also regulates expression of the E2F8 targets *Racgap*, *Ect2* and *Mklp1*. Bi-nucleation can also be induced

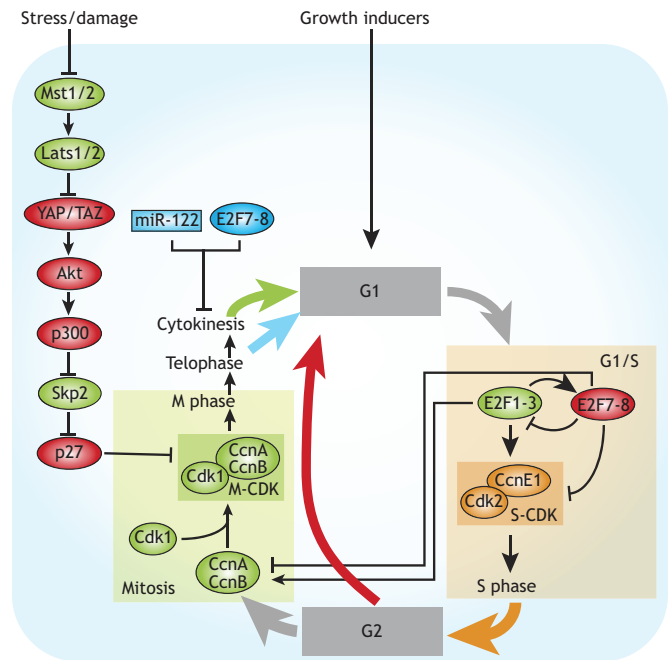


Fig. 6. Polyploidization during liver regeneration. Hepatocyte growth rates affect the activation of E2F1-3, which controls G1/S through transcriptional activation of cyclin E1 (CcnE1)/Cdk2. E2F1-3 also activate expression of E2F7-8, which in turn repress expression of E2F1 and its targets, thus forming a negative-feedback loop. E2F1-3 are also required for expression of CcnA and CcnB, which activate Cdk1 (M-CDK) and are required for M-phase entry. In hepatocytes, E2F1-3 depletion promotes endoreplication (red arrow), whereas E2F7-8 depletion promotes mitosis (green arrow). Endoreplication is also induced through the Hippo pathway. The Hippo homologs Mst1/2 suppress activity of the Yorkie homologs YAP and TAZ through activation of Lats1/2. Upon Mst1/2 inactivation, YAP promotes Akt activity, which promotes activation of the acetyl transferase p300. The subsequent p300-dependent acetylation of Skp2, an F-box protein of the SCF ubiquitin ligase complex, sequesters Skp2 to the cytoplasm. This prevents proteasomal degradation of p27, an inhibitor of M-CDK activity. Hepatocytes also become polyploid via endomitosis (blue arrow) through downregulation of cytokinetic regulators such as Rho-GTPase. E2F7-8 and miR-122 are known suppressors of cytokinesis and thereby promote endomitosis.

in cultured hepatocytes through stimulation by the cytokine TGF β , which prevents midzone localization of RhoA-GTPase, a known regulator of cytokinesis (De Santis Puzzonia et al., 2016). Furthermore, the insulin-PI3K-Akt pathway has been shown to control bi-nucleation, possibly through TORC2-dependent regulation of Rho-GTPase activity (De Santis Puzzonia et al., 2016).

Once hepatocytes reach a ploidy of 8C, they enter senescence through upregulation of p16^{ink4A}, p21 and p53 (Wang et al., 2014). However, senescence is reversible and polyploid hepatocytes may re-enter mitosis, giving rise to tetraploid and diploid cells, a dynamic process referred to as a 'ploidy conveyor' (Duncan et al., 2010). This suggests that senescent hepatocytes are programmed to allow re-establishment of M-CDK activity upon cell cycle entry. Ploidy reduction is prone to multipolar spindle formation during mitosis, and is therefore likely to give rise to aneuploid hepatocytes. However, hepatocytes manage to turn aneuploidy to the benefit of liver resilience, as aneuploidy creates a heterogeneous population of hepatocytes, some of which have increased fitness during chronic stress (Duncan et al., 2012a, 2010). Experiments in mice have revealed that livers with a heterogeneous population of hepatocytes can develop resistance against chronic injury through conditional cell selection, which gives rise to a less heterogeneous population of

hepatocytes that share genotypes that endow specific stress resistances (Duncan et al., 2012a,b, 2010).

The multiple modes of polyploidization observed in the liver illustrate how plastic the cell cycle can be. Mitosis, endomitosis and endoreplication all require cell cycle entry followed by DNA replication through S-CDK activity. Blocking cytokinesis through miR-122 leads to endomitosis, whereas blocking M phase altogether, through M-CDK repression, gives rise to endoreplication. In contrast to TGCs and *Drosophila* polyploid cells, where the cell cycle type seems ‘hard wired’ through strict downregulation of M-CDK activity, hepatocytes appear to adopt a plastic mode of cell cycle regulation. M-CDK activity in hepatocytes is not completely squelched, as in *Drosophila* endocycling cells, but appears to be dampened and adjusted to various levels through CKIs and E2F7/8 activity. Moreover, a study in HeLa cells has shown that high concentrations of CDK1 inhibitors induce endocycles, whereas lower concentrations induce endomitosis (Chen et al., 2016), suggesting therefore that levels of M-CDK activity determine whether a cell performs mitosis, endomitosis or endocycling in an activity level-dependent manner. We therefore presume that promiscuous regulation of CDK in the liver allows sufficient M-CDK activity for sister chromatin separation and karyokinesis in endomitotic hepatocytes, whereas M-CDK activity is sufficiently restrained to block APC/C^{cdc20} activation and anaphase in endoreplicating hepatocytes. Interestingly, cytokinesis and M-CDK activity appear to be regulated by some of the same upstream regulators (e.g. Akt, E2F8/7), and thus we speculate that the M-CDK/RhoGTP balance may control cell cycle decisions in the mammalian liver.

Conclusions and perspectives

In animals, the capacity for regeneration is often determined by the presence of mitotically capable stem cells, which can provide new cells as needed. This is typical in tissues with a high turnover rate, such as those exposed to harsh environments, like the intestine and skin. Cells in other tissues, however, are made to last and may be supported by few or even no stem cells. In extreme cases, cells that form a tissue during development must support tissue/organ function for the life of the organism, as is the case of human cardiomyocytes (Tzahor and Poss, 2017), the mammalian central nervous system and many organs in short-lived invertebrates. But what underlies these differences in regenerative modes and capacities? One possibility is that tissue function is the underlying factor. Cardiomyocyte function, for example, requires continuous contractile activity generated by actin-myosin sarcomeres. Mitotic proliferation requires disruption of sarcomeres, which would temporarily compromise cardiomyocyte function. Polyploidy in the heart may thus be beneficial because of acquired resistance to apoptosis, which ensures longevity, and because it allows growth while maintaining tissue function. In fact, polyploid cells, such as mammalian TGCs and *Drosophila* subperineurial glia, often form barrier tissues where growth of such barriers can be maintained continuously, without the loss of cell-cell junctions that occurs during mitosis (Unhavaithaya and Orr-Weaver, 2012; Von Stetina et al., 2018). However, polyploidization is not only an asset in wound healing; it can enhance cellular damage resistance. The protective cells that cover the skin, keratinocytes, undergo endomitosis and endocycling to a maximum ploidy of 12C (Gandarillas and Freije, 2014). Polyploidization of keratinocytes is induced by UV irradiation and is likely to increase tolerance to genotoxic stress (Gandarillas, 2012; Gandarillas et al., 2018), as reported in hepatocytes (Zhang et al., 2018). In fact, in plants, strains with increased ploidy are more resistant to UV radiation (Gegas

et al., 2014). Similarly, the polyploid tissues of *Drosophila* larvae can survive high doses of irradiation that are sufficient to kill mitotic progenitor cells (Hassel et al., 2014; Zhang et al., 2014). As mentioned here, there seem to be several advantages to polyploidization in tissues where it is pre-programmed. It would thus be interesting to explore the extent to which these advantages can be conferred to ectopically induced polyploid cells.

We have learned a lot about how polyploidy is generated through endoreplication and cell-cell fusion, but there is still more to understand, for example about the damage sensors that induce regeneration through either mitosis or endoreplication. As discussed, several recent examples demonstrate the involvement of the Hpo pathway in the wound healing response. In mammals, the Hpo pathway is involved in contact inhibition of cell proliferation, where low cell density appears to lower the threshold for growth factor-sensitive proliferation (Gumbiner and Kim, 2014). This makes sense as a signal during the wound repair process, because cells adjacent to a wound have fewer cell contacts, and this condition would thus promote proliferation through the Hpo/Yap pathway. Tissue injury and cell death are also associated with ROS production, which stimulates JNK activity, and appear to have important roles in damage sensing (Mittal et al., 2014; Santabarbara-Ruiz et al., 2015). Further exploration of the primary damage sensors should be of great interest in fields involving regeneration.

As seen in mammalian hepatocytes, zebrafish cardiomyocytes and *Drosophila* epithelial cells, the generation of multinuclear cells during regeneration is a recurrent mechanism in wound healing. The advantage of multinuclear cells over mono-nuclear polyploid cells is not clear. Perhaps the added genome-to-nuclear surface ratio of multinuclear cells, or the dispersal of nuclei in a large cytoplasmic space, may be advantageous to cell growth and/or function. As reviewed here, recent studies of regeneration have highlighted the involvement of polyploid cells, but there are still many questions to be answered about how polyploidy is elaborated during wound healing, and what its advantages are. A potential future application of artificially induced polyploidy is to enhance the regeneration of postmitotic tissues that lack stem cells, for example during recovery from myocardial infarction. We are rapidly gaining the tools needed to trigger polyploidization, a simpler process than restoring the entire mitotic proliferation program, and it will be interesting to test these tools in various wound-healing and regeneration scenarios.

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Competing interests

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