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Solving the polyploid mystery in health and disease

Gjelsvik KJ¹, Besen-McNally R¹, and Losick VP^{1,*}

¹MDI Biological Laboratory, 159 Old Bar Harbor Rd., Bar Harbor, ME 04609.

Abstract

Polyploidy (the more than doubling of a cell's genome) frequently arises during organogenesis, tissue repair, and age-associated diseases. Despite its prevalence, major gaps exist in how polyploid cells emerge and affect tissue function. Studies have begun to elucidate the signals required for polyploid cell growth as well as the advantages and disadvantages of polyploidy in health and disease. This review highlights the recent advances on the role and regulation of polyploidy in *Drosophila* and vertebrate models. The newly discovered versatility of polyploid cells has the potential to provide alternative strategies to promote tissue growth and repair, while limiting disease and dysfunction.

Keywords

Polyploidy; Endoreplication; Aneuploidy; Tissue Repair; Hippo; Mechanotransduction

A **polyploid** cell is defined as a cell that contains more than the diploid copy of its chromosomes. The total DNA content is represented by the amount of 'chromatin' in the cell, known as the **C-value**. Haploid cells (i.e. sperm) are 1C, diploid cells are 2C, and polyploid cells therefore are at least 3C or greater. Cells are either developmentally programmed to become polyploid or opt to adopt the polyploid fate as a stress resistance response. This review focuses on the recent observations of polyploidy in *Drosophila* and vertebrates (mice and zebrafish) that has given new insights into the functional significance and regulation of polyploidy in health and disease.

Generating a polyploid cell

Polyploid cells are generated by cell cycle dependent or independent mechanisms (Figure 1). The mitotic cell cycle depends on four sequential phases: G1-S-G2-M which is driven by cyclin-dependent kinases (CDKs). CDK-cyclin activation allows cells to progress through the canonical phases of the cell cycle. For example, CDK2-cyclin E complex drives S phase (DNA replication) and CDK1-cyclin B complex drives M phase, in which chromosomes are segregated and the cell undergoes cytokinesis resulting in two daughter cells (Figure 1A).

*Corresponding Author: vlosick@mdibl.org.

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Endoreplication encompasses two types of incomplete cell cycles: **endocycle** and **endomitosis** [1]. Endomitosis refers to a cell cycle that initiates but does not complete M-phase (Figure 1C). This is either due to a block in cytokinesis resulting in a binucleated, polyploid cell or truncates M phase, before telophase, resulting in a mononucleated, polyploid cell (Figure 1C). In the endocycle, a cell replicates its genome in S phase, but completely bypasses M phase with alternating G and S phases (Figure 1D). Inhibition of cell's mitotic machinery enables M phase to be skipped over. This often occurs by targeting mitotic cyclins for proteolytic degradation via the anaphase-promoting complex/ cyclosome (APC/C) E3 ligase or inhibition of CDK-cyclin complex activity by CDK inhibitors (CDI). The oscillation of CDK2-cyclin E activity from low to high in G to S phase allows cells to initiate sequential endocycles [2]. Each endocycle will double the cell's genome allowing a cell to reach C-values as high as 200,000C [3]. Alternatively, bi- and multinucleated, polyploid cells can also form by cell fusion, which is not dependent on the cell cycle (Figure 1E).

Distinguishing diploid vs polyploid growth and division

A major challenge in the tissue growth and regeneration field has been to faithfully distinguish between polyploidization and proliferative cell cycle events. The classical cell cycle markers, including S phase markers (PCNA, ³H-thymidine, EdU, or BrdU) do not distinguish endoreplication from a mitotic cell division, since in both cases cells will enter S phase (Figure 1A-1D). Likewise, the M phase marker, phospho-histone H3 (pH3), will not differentiate endomitosis and mitotic cell division, as chromosome condense during metaphase and are readily labeled with pH3 in both cell cycles [4]. The Ki67 marker labels all phases of cell cycle and only distinguishes cycling cells from post-mitotic cells [5]. Polyploid cells, in some circumstances, have the capacity to divide so cytokinesis markers are also not reliable (Figure 1B) [6, 7]. In fact, no cell cycle marker exists that can distinguish a cell division that will result in a diploid versus polyploid cell (Figure 1A and 1B).

More sophisticated strategies are now being employed to conclusively detect a complete mitotic cell cycle resulting in cytokinesis and generation of two diploid daughter cells versus endoreplication to generate polyploid cells. One strategy is to use single-cell analysis either by imaging fixed cells, fluorescence-activated cell sorting (FACS), or single cell DNA sequencing [8–10]. These methods allow a cell's nuclear DNA content (ploidy) to be measured, a rigorous method to distinguish between diploid (2C) and polyploid (>3C) cells. Single cell DNA sequencing has additional advantage of being able to detect chromosome copy number variation thereby detecting **aneuploidy** [10]. Another strategy is to use live imaging to follow cell division and growth *in vivo* [11]. These methods in combination with fluorescent reporters that allow all phases of cell cycle including cytokinesis to be detected [12, 13]. This has led to a significant recognition of the prevalence of polyploidy in development, regeneration, and disease. Long-held views of how tissues grow and repair are being put into question using these emerging techniques.

Polyploidy: a common strategy for cellular differentiation and organogenesis

Polyploid cells are ubiquitous in plants and insects and have been well studied in these model organisms, *Arabidopsis* and *Drosophila* [14, 15]. In mammals, polyploidy was thought to be unique to only a few cell types, most notably the placenta giant trophoblasts (up to 512C), megakaryocytes (up to 128C), and liver hepatocytes (up to 16C) [14]. However, advances in imaging with fluorescent cellular markers has allowed intact mammalian tissues to be visualized enabling the detection of remarkable number of polyploid cells in nearly all mammalian organs. These new imaging tools enabled the identification of binucleated secretory alveolar cells in the lactating mammary gland [16]. The secretory alveolar cells become polyploid by endomitosis (also referred to as failed cytokinesis), which is required for milk production (Figure 2B) [16].

It is becoming appreciated that many mammalian tissues are composed of >50% polyploid cells as it is part of their developmentally programmed differentiation [17–21]. The switch to polyploidy often coincides with the onset of postnatal life. What still remains more poorly understood is functional significance of polyploidy. The atypical E2Fs (E2F7 and E2F8) regulate polyploidization by inhibition of mitotic gene expression, a requirement for the endocycle [18, 19]. However, conditional knockout of E2F7 and/or E2F8 in liver hepatocytes and pancreas exocrine and endocrine cells has no apparent consequence to physiological organ function despite affected cell types being diploid, instead of polyploid [18, 19, 21]. In liver, two recent studies have now revealed that polyploid hepatocytes help to prevent tumor growth (Figure 2F) [22, 23]. While polyploidy may be dispensable for organ development in some cases, it's appears to offer an adaptive advantage for disease resistance during adult mammalian life.

Polyploid cell generation in organogenesis

A variety of mechanisms are used to generate polyploid cells, but what drives polyploid cells to form by one strategy versus another is an open question. In the blood, megakaryocytes develop by endomitosis forming multinucleated polyploid cells required for platelet production [14]. Remarkably, megakaryocytes can be reprogrammed into an endocycle by genetically ablating the mitotic regulator, CDK1, with no inhibition of platelet formation [24]. This is an example where polyploidy generation is functionally interchangeable, but this is not always the case. The *Drosophila* blood brain barrier (BBB) was found to be composed of both mono- and multinucleated polyploid subperineurial glia (SPG), which are generated by the endocycle and endomitosis during larval development (Figure 2A) [25]. Notch signaling promotes the endocycle but inhibits endomitosis similar to its known role in mitotic to endocycle switch in the *Drosophila* intestinal and follicle epithelial cells [15, 25, 26]. Genetically manipulating Notch signaling or mitotic entry can shift the balance of mono- and multinucleated SPG cells but doing so impaired integrity of the BBB [26]. Therefore, in this tissue, mono- and multinucleated cells are not interchangeable and both endocycle and endomitosis are required for the development of a functional barrier. The advantages and constraints of making mono- versus multi-nucleated polyploid cells still remains to be elucidated. It may be a matter of cell size as the multinucleated SPG cells

were found to attain a larger cell size despite having the same ploidy as their mononucleated counterparts [26].

Polyplloid cell division in organogenesis

Polyplloid cell division is unusual as most polyplloid cells are terminally differentiated and unable to return to mitosis. This is in part because polyplloidy poses a challenge to cell division since dividing polyplloid cells readily accumulate mitotic errors, including chromosome aberrations and aneuploidy associated with cancer cells (Figure 2E) [27, 28]. Despite these constraints, mouse liver hepatocytes and *Drosophila* rectal papillar cells retain the capacity to divide [6, 7, 29]. These models of polyplloid cell division are providing new insights into how polyplloid division is regulated with the risk of aneuploidy.

Drosophila's rectal papillar first endocycle to become octoploid and then mitotically divide two times during development [6]. To retain mitotic competence, a unique endocycle program, including the retention of centrosomes and late replicating genomic regions, was discovered [30]. In addition, the polyplloid papillar cell chromosomes are **polytene**, a state in which chromosome copies are bound within their chromatids. Frequent mitotic errors were found to be reduced by dissociating the polytene chromosomes into separated chromatid pairs prior to anaphase, named separation into recent sister chromatid pairs (SIRS) [31]. Inhibition of SIRS, by genetic loss of Mad2, delays SIRS resulting in significant increase in chromosome aberrations and aneuploidy after polyplloid cell division [31].

In liver, polyplloid hepatocytes also retain mitotic competence and were reported to generate aneuploid cells during development and homeostasis [7, 29]. However, the high percentage of aneuploid hepatocytes in the normal liver has been put into question using the single cell DNA sequencing method [10, 29]. Strikingly, it was found that the tissue environment dictates a polyplloid cell's susceptibility to aneuploidy during division. Dissociated polyplloid hepatocytes grown in culture frequently become aneuploid, whereas polyplloid division of hepatocytes within developing neonatal liver retained the capacity to accurately segregate chromosomes [10, 33]. In adult liver regeneration, the tissue architecture is also disrupted and aneuploidy arises in the dividing polyplloid hepatocytes [7, 29]. Still it remains to be determined whether aneuploid hepatocytes persist or are eliminated after liver regeneration completes as aneuploidy can activate the immune response [33]. What would be the consequence if aneuploid cells persisted? A growth advantage by resulting in genetic diversity in liver or as an initiator of disease progression [28, 32, 34].

Polyplloidy: the benefits and limits for tissue repair

Polyplloidy as a driver of tissue repair

Many adult tissues lack a resident stem cell population and are solely composed of terminally differentiated cells that lack the capacity to proliferate. As a consequence, polyplloidy serves as an alternative strategy to promote wound repair and compensate for cell loss when cell division is restricted. Recently, polyplloid cell growth has been found to contribute to tissue repair and regeneration in several *Drosophila* and vertebrate tissues. In *Drosophila*, the adult abdominal epithelium heals puncture wounds by wound-induced

polyploidization [8, 35, 36]. Enlarged multinucleated, polyploid cells form by cell fusion and endoreplication, which function simultaneously to re-establish the adult fly epithelial barrier (Figure 1D, 1E, and 2C) [35]. In *Drosophila* egg development, follicular epithelial cells switch from mitotic cycle to endocycle. Genetic ablation of the follicle cells or a reduction in follicle cell growth triggers an insulin-dependent compensatory cellular hypertrophy [37]. As result, the follicular epithelial cells compensate for cell loss by boosting the endocycle to generate epithelial cells with higher ploidy [37].

Many tissues utilize both polyploidization and cell proliferation to heal. The regenerative capacity of the mammalian liver relies on the ability of hepatocytes to polyploidize as well as divide as either diploid or polyploid cells [38]. Hepatocytes cell growth was found to precede hepatocyte division following surgical removal the liver mass [38]. The *Drosophila* gut relies on both stem cell proliferation and endoreplication of daughter cells to maintain homeostasis and regenerate [39]. Polyploid enterocytes, the major cell type of the fly intestine, differentially respond to physiological demands of the organ by boosting cell growth via the endocycle. InR, Pi3K, and Tor signaling activate the endocycle during homeostasis, whereas tissue damage activates EGFR, Ras, and MAPK signaling to induce polyploid growth of the enterocytes [39].

Polyploid cells can also be a source for the generation of new intestinal stem cells. Under conditions of severe stem cell loss, polyploid (4C) enteroblasts, the immediate stem cell daughter, are capable of undergoing a process of reductive cell division known as **amitosis** [40]. The reductive division of the polyploid enteroblasts into two diploid daughter cells enabled the fly intestinal stem cell pool to be restored [40]. Reductive polyploid divisions have also been observed during mouse liver regeneration, suggesting the amitosis may be conserved strategy to resupply the organ with a diploid cell pool that has more proliferative potential [7, 32, 38].

In vertebrates, wound-induced polyploidization has also been discovered to play a beneficial role in kidney and heart repair [11, 41]. The mouse kidney is capable of withstanding acute injury as tubular cells were thought to be replaced by de-differentiation and cell division [42]. However, a recent study has put this model into question by taking advantage of combination of cellular and genetic tools, including a tubular cell-lineage analysis, the FUCCI cell cycle reporter, and ploidy analysis [41]. As a result, tubular epithelial cells were found to endocycle in response to acute kidney injury and polyploid cell growth of tubular cells helped to restore renal function [41].

In the zebrafish heart epicardium, live cell imaging, unexpectedly, revealed that large, multinucleated polyploid epicardial cells form during heart regeneration (Figure 3) [11]. Both the endocycle and endomitosis contribute to generation of polyploid epicardial cells. However, after heart regeneration concluded the polyploid cells were cleared by apoptosis and replaced by dividing epicardial cells [11]. The upregulation of mechanical tension at the leading edge was found to be sufficient to drive endoreplication, suggesting that specific mechanical inputs could favor polyploid cell growth, instead of diploid cell division (Figure 3) [11]. Mechanical stimuli are known to be required for tissue morphogenesis during

development by controlling cell proliferation, but the biophysical cues instructing polyploid cell growth in development and disease remain to be identified.

Polyploidy as a barrier to tissue repair

Damage to the adult mammalian heart results in permanent loss of myocardium and formation of fibrotic scar tissue that impairs heart function and increases the risk of heart failure [43]. The block in heart regeneration correlates with the onset of cardiomyocyte polyploidization at mouse postnatal day 7 [20, 44]. The cardiomyocytes are capable of limited cell cycle activity, where polyploidization predominates, yet is not sufficient to restore myocardial mass post injury [20, 44, 45]. In contrast, the adult zebrafish regenerates its myocardium through proliferation of preexisting diploid cardiomyocytes [46]. Genetically inducing cardiomyocyte polyploidization in zebrafish heart also impaired regeneration [47, 48]. Likewise, genetically selecting for inbred mouse strains with higher frequency of diploid cardiomyocytes was found to improve heart functional recovery after coronary artery ligation [48]. Taken together, cardiomyocyte polyploidization appears to be a barrier for heart repair. It is surprising that polyploidy acts as both a driver and repressor of tissue repair depending on the tissue/ organ context. For lost tissue mass to be regenerated, polyploid cells must retain the capacity to endoreplicate or divide (Figure 1B-1D). It remains to be determined if the impaired regenerative capacity of polyploid cardiomyocytes is due to the inhibition of polyploid growth and/ or division.

Restoring organ size via polyploid cell growth

The regulation of organ size (or mass) is essential to generating a functional and correctly proportioned tissue for both tissue growth and regeneration. A master regulator of organ size in both flies and mammals is the Hippo signal transduction pathway that allows tissues to grow during development and regrow during repair to defined sizes [49, 50]. Hippo pathway was originally discovered to control tissue growth by regulating cell division through Yki/ Yap (*Drosophila*/ mammalian) dependent transcription of cell survival and cell cycle genes [51]. It is now appreciated that Yki/ Yap also regulates endoreplication, thereby controlling organ size by regulating cell size.

In *Drosophila* development and wound repair, entry into the endocycle is dependent on Yki [8, 52]. The Yki-dependent induction of S phase genes induces entry into cell cycle, but expression of E3 ligase Fizzy-related (Fzr) bypasses M phase resulting in endocycle instead of mitosis (Figure 1D) [52, 53, 54]. In mice, Yap promotes polyploid cell growth by regulating Skp2, an E3 ligase that targets the CDI p27 for proteolytic degradation [55]. The accumulation of p27 causes hepatocytes to default into an endocycle and boost ploidy [56]. The regulation of polyploid cell growth by Hippo signaling is a crucial, but a more poorly understood factor in organ size control. It remains to be determined whether mechanical or other signals regulate the Hippo pathway to instruct the extent of polyploid growth required to develop and regrow tissues after injury.

These studies have revealed that a key detriment of whether polyploidy is used for tissue growth and repair is decided by the cell's mitotic competence. Therefore, how feasible is it to switch between polyploid cell growth and diploid cell division? Some cell types have

plasticity and can be switched by genetic or pharmacological regulation of the cell cycle [11, 54, 56, 57]. Remarkably, polyploid cell growth is sufficient to restore tissue mass without relying on cell division (Figure 4) [8, 11, 54, 56, 57]. However, switching mechanisms of tissue repair may not be feasible for all cell types. Genetically forcing adult *Drosophila* epithelial cells to divide impaired wound healing demonstrating that polyploid cell growth, in some tissues, may be the only tolerated mechanism to heal [53]. There may also be long-term detrimental consequences to switching from polyploidization to proliferative growth as mouse liver and *Drosophila* hindgut were found to be more sensitive to tumor growth upon oncogenic activation [22, 23, 54].

Role of polyploidy in age-associated diseases

Polyploidization is known in many mammalian organs to increase with age, including in the liver, heart, brain, and eye [43, 58–60]. Polyploidy may accumulate with age due to the decline in cell proliferation and resident tissue stem cell loss. The increase in polyploidization with age could function as either a beneficial tissue repair strategy or a driver of disease. In the mammalian eye, cornea endothelial cells are frequently lost with age and disease including, Fuchs endothelial corneal dystrophy (FECD) [60, 61]. The surviving endothelial cells enlarge in size and often become multi-nucleated (Figure 2D). In a mouse model of FECD, polyploidization was found to accompany the enlargement of cornea endothelial cells [8]. Like in *Drosophila* and vertebrate models, polyploid endothelial cells precisely compensated for the endothelial cell loss, as total tissue ploidy did not change despite reduced endothelial cell number (Figure 2D and Figure 4) [8, 11, 54, 56, 57]. Therefore, corneal endothelial polyploidization serves as an example where age-associated polyploidy promotes repair by compensating for cell loss.

Polyploidy is more often than not associated with many age-associated diseases, including cancer. The discovery that tetraploidy predisposes cells to aneuploidy and tumor formation upon cell division, along with reports that most cancer cells are in a polyploid state has created renewed interest in polyploidy's role in tumorigenesis [27, 28, 62]. The hallmarks of cancer cells, including DNA damage resistance and aneuploidy, are known traits that polyploid cells have acquired for physiological function.

Polyploid cells alter their genome copy number as regions of the genome have been found to be either amplified or underreplicated during the endocycle in both *Drosophila* cells and mouse giant trophoblast cells [63–67]. The re-replication of genome causes DNA damage due to stalled replication forks, yet p53-dependent DNA damage response is epigenetically silenced during polyploid growth [68–70]. The division competent rectal papillar cells are also able to divide in the presence of DNA damage as alignment and segregation of acentric chromosome fragments occurs via the Fanconi anemia protein, FANCD2 [71]. By becoming polyploid, cancer cells may exploit the survival and growth mechanisms that polyploid cells have adapted thereby driving tumorigenesis and disease.

Concluding Remarks and Future Perspectives

Polyploidization is a conserved part of our tissues' ability to develop, grow, and heal. In the last five years, the mystery of polyploid function in health and disease has begun to be unraveled. Polyploid cells provide many advantages over their diploid counterparts. Polyploidy allows cell to grow by orders of magnitude, which is critical for tissue development and maintenance of tissue integrity. Final organ size can also be reached by polyploid cell growth and division. As a result, animal tissues can grow and regrow with fewer, yet larger cells. Conserved signal transduction pathways regulate endoreplication, but how a cell senses how big to grow remains unknown (see **Outstanding Questions**). Mechanical cues from the tissue environment instruct cell proliferation and may also direct polyploidization through inactivation of the Hippo signal transduction pathway. In addition, polyploid cells possess unique traits (DNA damage resistance and genome copy number variation), which may be exploited to drive cancer and other diseases. Continued research on the role and regulation of polyploidy in health and disease will uncover the versatility of polyploid cells to support life. In the future, the goal will be to exploit the advantages of polyploidy to improve our ability to heal and prevent disease, while identifying the mechanisms that restrict polyploidy when disease and tissue dysfunction arise.

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Glossary Box:

Aneuploidy	The cell's total chromosome number is either increased or reduced compared to the biological number of chromosomes in the organism
Amitosis	A cell division that occurs without spindle formation by cleavage of nucleus and division of the cytoplasm
C-value	The total DNA content of a cell as measured by the total chromatin content. Haploid cells (i.e. sperm and egg) are 1C and diploid cells are 2C
Endocycle	The process by which a cell replicates its genome through alternating G and S phases, but completely bypasses M phase of the cell cycle. Each endocycle doubles the C-value
Endomitosis	A cell cycle that initiates but does not complete mitosis either due to a block in cytokinesis or earlier truncation of M phase
Endoreplication	An incomplete cell cycle that replicates DNA during the S phase without the completion of mitosis or cytokinesis
Polyploidy	A cell that has more than diploid copy of its genome. Polyploid cells arise by cell cycle dependent and

independent mechanisms thereby generating both mono- or multinucleated cells

Polytene

Large chromosomes composed of many copies of the DNA strands bound together by repeated rounds of DNA replication in the endocycle

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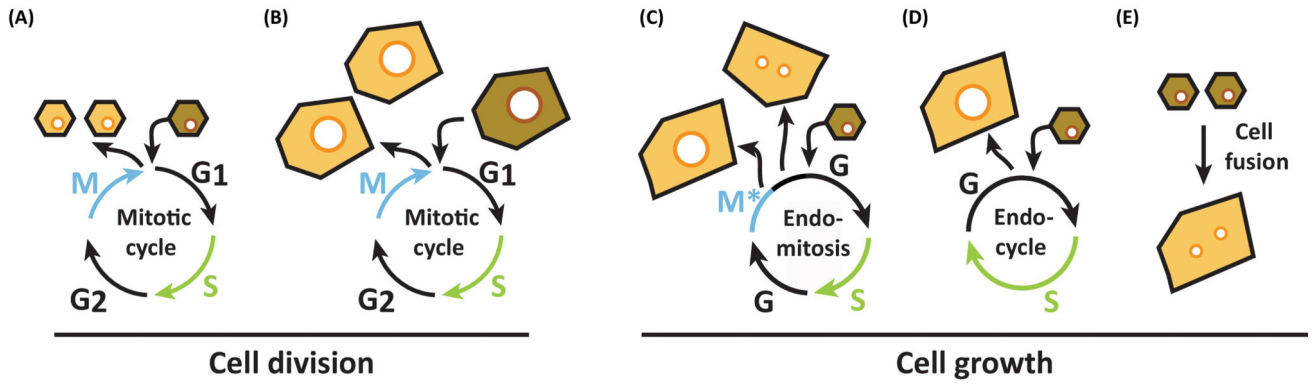
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Highlights:

- Advances in imaging and fluorescent cellular markers has revealed that polyploidization is a common strategy for tissue growth and repair in many organisms and organs.
- Polyploid cells grow in size via endomitosis, the endocycle, and/ or cell fusion, but can also retain the capacity to divide.
- Hippo signaling and mechanical forces regulate polyploid cell growth to maintain and restore organ size.
- Depending on the context, polyploidy either promotes repair and maintains homeostasis or impairs regeneration and drives disease.

Outstanding Questions:

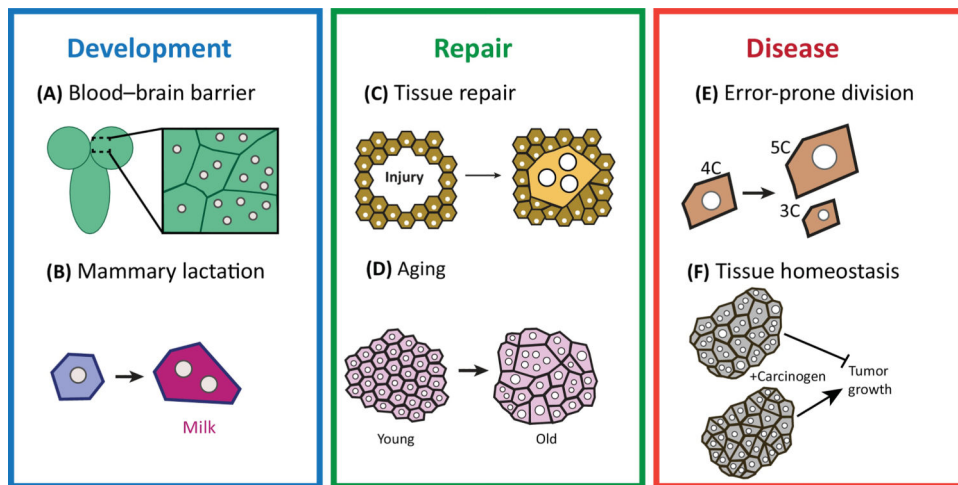
1. How do cells decide to fuse or endoreplicate to generate a polyploid cell? Are there advantages and limitations of mono- and multinucleated polyploid cells for tissue function?
2. What determines whether polyploidy is a barrier or driver of tissue repair? Does cell cycle competence dictate the regenerative capacity of polyploid cells?
3. Does mechanical stimuli signal through Hippo pathway to regulate polyploid cell growth and control organ size? Do specific mechanical inputs favor polyploid cell growth, instead of diploid cell division?
4. How does polyploidy alter long-term tissue function, when polyploid cells arise in response to injury?
5. When polyploid cells arise with age do they serve to promote tissue repair or as a source for disease initiation?



Trends in Genetics

Figure 1. Generation of diploid and polyploid cells through division and growth.

The mitotic cell cycle generates either two diploid daughter cells (A) or two polyploid daughter cells (B) depending on whether the initiating cell is diploid (A) or polyploid (B). Incomplete cell cycles promote polyploid cell growth by either endomitosis (C), which results in a mono- or binucleated, polyploid cell or via the endocycle (D) resulting in a mononucleated polyploid cell. (E) Cell fusion also generates bi- and multinucleated polyploid cells independent of the cell cycle. Cell cycle markers do not discern polyploid vs diploid cell generation. A cell's total ploidy (DNA content) has to be measured to rigorously distinguish between diploid (A) and polyploid (B-E) cell outcomes.



Trends in Genetics

Figure 2. The functional significance of polyploidy in health and disease.

Illustrations of recent discoveries of polyploidy in health and disease. (A) Development and function of the blood brain barrier in *Drosophila* requires a balance of mono- and multinucleated polyploid cells generated by endocycle and endomitosis. (B) Mammary gland lactation requires polyploidization of secretory cells by endomitosis. (C) Wound-induced polyploidization occurs in *Drosophila* and vertebrate tissues to promote repair. (D) Polyploid cells arise with age and may help to compensate for cell loss. (E) Polyploid cells can retain the capacity to divide yet are susceptible to error-prone division resulting in aneuploidy. (F) Polyploid cells can also restrict tumor growth.

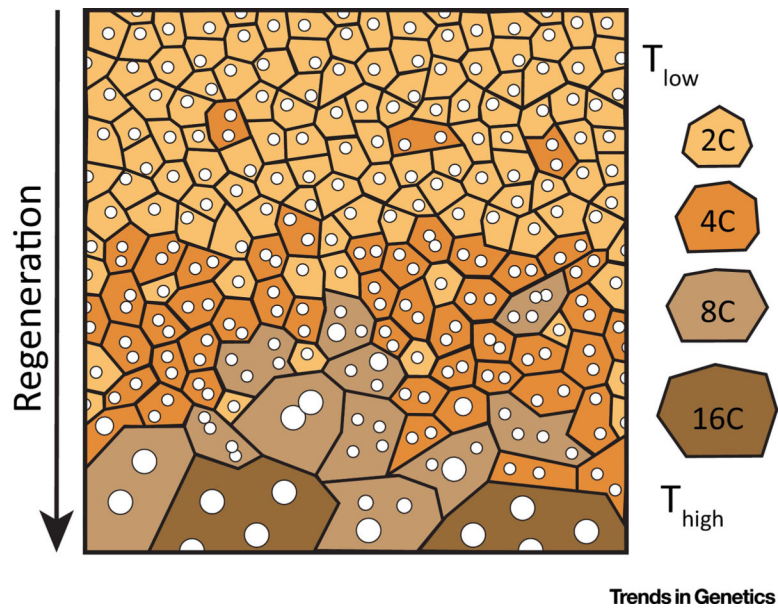


Figure 3. Mechanical tension is a driver for polyploid cell growth.

The zebrafish epicardium heals by cell division and polyploidization. The tissue is composed of mostly diploid cells (2C) prior to injury. During regeneration, higher tension (T_{high}) occurs at the leading edge and is sufficient to induce polyploid growth by boosting the endocycle and endomitosis.

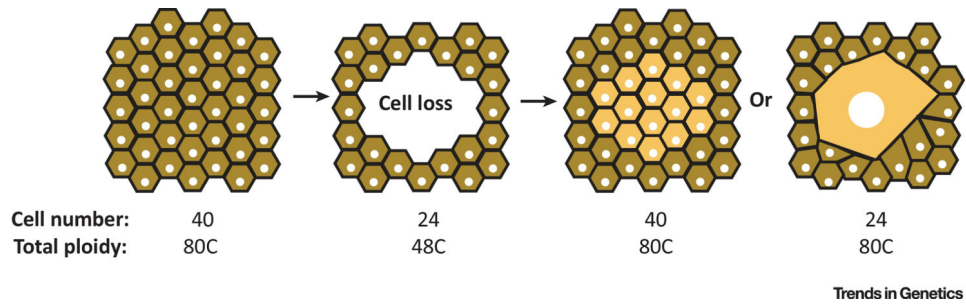


Figure 4. Organ size (mass) is determined by cell number or cell size.

Model illustrates how organ mass is restored by either cell proliferation (cell number) or polyplodization (cell size) following cell loss caused by various tissue insults, including injury or cell turnover as result of aging and disease. Polyplodization allows organ mass to be restored with fewer total cells as total ploidy of tissue remains unchanged.