When bigger is better: the role of polyploidy in organogenesis

When Bigger Is Better: The Role of Polyploidy in Organogenesis

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Abstract

Defining how organ size is regulated, a process controlled by not only the number of cells but also the size of the cells, is a frontier in developmental biology. Large cells are produced by increasing DNA content or ploidy, a developmental strategy employed throughout the plant and animal kingdoms. The wide-spread use of polyploidy during cell differentiation makes it important to define how this hypertrophy contributes to organogenesis. I discuss here examples from a variety of animals and plants in which polyploidy controls organ size, the size and function of specific tissues within an organ, or the differentiated properties of cells. In addition, I highlight how polyploidy functions in wound healing and tissue regeneration.

Polyploidy: Going big

Organogenesis, the formation of organs during development, involves determination and differentiation of the cell types necessary for organ function and proper arrangement of these cell types into tissues. A second crucial but poorly understood aspect of organogenesis is regulation of size. Not only must the overall size of the organ be controlled, but each of the tissue layers within the organ needs to be scaled to be the proper size. Tissue and organ size are dictated by the sum of cell number and cell size. Removal of sections of mammalian liver, imaginal discs in Drosophila, or the endosperm in plants, either by physical or genetic manipulation, reveals a mechanism to monitor total organ mass, because in all these cases normal organ size is restored [1–3]. The mechanisms by which total organ mass is monitored are unknown, as are those by which tissues measure their size with respect to other tissues in the organ.

In many plant and animal organs size is controlled at the level of cell number. Thus organ size and tissue scaling largely are affected by regulation of cell proliferation. Here I discuss a second strategy to regulate size, which involves the generation of large cells by increased DNA content, or polyploidy. Diploid cells can double their size by growth [4, 5]. In many

developmental contexts, however, cells increase in size by orders of magnitude. The generation of such large cells is invariably associated with increased DNA content [6]. Nuclear volume and total cell size scale with DNA content, and the polyploidy present in large cells indicates there is a minimal nuclear to cytoplasmic ratio throughout biology [7]. A complete understanding of organ and tissue size therefore requires delineating the regulation of cell polyploidization in development and its coordination with cell proliferation and differentiation.

OVERVIEW OF SOMATIC POLYPLOIDY

The role of polyploidy in development is distinct from species ploidy, as it involves increased DNA content in specific somatic cell types during development. This is in contrast to organisms, such as many plants, in which a polyploid genome content is transmitted through the germline, resulting in every cell in the body being polyploid. Somatic polyploidy of specific tissues within an organism is sometimes termed endopolyploidy, but here I refer to it solely as polyploidy. Somatic polyploidy is defined by extra copies of the DNA for all the chromosomes, thus differing from aneuploidy in which the copy number of only one or a few chromosomes is changed.

Polyploid cells can result from variant cell cycles or cell fusion. Cell fusion is important in the generation of skeletal muscle cells, but will not be discussed here. Distinct cell cycle variants produce mono or multinucleate polyploid cells (Figure 1, Glossary). The endocycle contains solely a gap (G) phase during which gene expression and growth occur and a DNA synthesis (S) phase [8]. In the endocycle all aspects of mitosis are shut off. The endocycle results in mononucleated cells with increased genome copies, but these can be arranged in two ways [6]. In polyploid cells the replicated chromosome copies are not physically aligned, and the DNA has an interphase appearance. In polytene cells the sister chromatid copies are attached to produce visible chromosomes with banded patterns. The endomitotic cycle retains steps of mitosis and can produce mono or multinucleate cells (Figure 1C). Endomitosis was originally defined as separation of replicated chromatids in the absence of nuclear envelope breakdown, but this occurs in only rare cases. This definition has been broadened to include cases such as megakaryocytes in which nuclear envelope breakdown and parts of anaphase occur without nuclear division, producing mononucleate cells with separated chromatids. Other cells such as hepatocytes undergo nuclear division but not cytokinesis, resulting in polyploid cells with multiple nuclei.

Polyploidy is a normal developmental pathway in many organisms, including mammals, insects, gastropods, and angiosperms. It can be a mechanism to increase total organ size when the majority or all of the cells in an organ are polyploid. This occurs in insects in which polyploid organs are prevalent. This has been investigated most extensively in Drosophila, in which nearly all of the differentiated larval tissues are polytene, and the organismal growth observed between the three larval stages is a consequence of increased cell size from increased ploidy rather than increased cell number [9]. Only the precursors for the adult organs and the nervous system undergo cell proliferation in Drosophila larvae. When these tissues differentiate during pupation, many produce polyploid adult organs. In addition to insects, in the chordate *Oikopleura dioica* most of the tissues become polyploid

[10]. The size of the tomato is dictated by the ploidy of cells of the pericarp, the fruit tissue [11, 12].

At the cellular level, several advantages have been proposed for large polyploid cells over a comparable mass of diploid cells: 1) large cells can act as a tissue envelope; 2) the absence of mitosis and cell division has been argued to permit polyploid cells to be more metabolically active [13]; 3) in contrast to the many diploid cells filling a comparable area through proliferation, each polyploid cell is essentially a homogenous clone. Thus, diploid cells can be plastic, as they can acquire different characteristics as they divide, whereas the polyploid cell can have a more stable, differentiated state; 4) multiple genome copies within a cell provide protection against mutations and thus damage resistance; 5) apoptosis is inactivated in at least some polyploid cells, possibly lengthening life span [14–16].

There are potential disadvantages to implementing polyploidy as a growth strategy. The clearest one is that as the volume of a spherical nucleus increases, the surface area does not keep up (spherical volume= $4/3\pi r^3$, but surface area=$4\pi r^2$). Thus processes such as nuclear export likely to depend on surface area of the nuclear envelope may become compromised. This may account for why many polyploid nuclei are flat or contain indentations throughout the nuclear envelope that increase surface area [12, 17]. An additional disadvantage appears in polyploid cells capable of resuming cell proliferation, such as mammalian hepatocytes and Drosophila rectal papillar cells [2, 18]. In these cases the presence of multiple chromosome copies, multiple centrosomes, and the absence of apoptosis cause frequent aneuploidy if polyploidization is followed by mitotic divisions. It has been hypothesized that in these cells polyploidy preceding mitosis may provide a mechanism for genetic variation in the final daughter cells [19].

Here I discuss examples from the plant and animal kingdoms in which cell size in tissue layers or specific cell types is controlled by ploidy and the apparent biological advantages (Table 1). Rather than an exhaustive list of all known polyploid cell types, I present representative examples of polyploidy being critical for tissue layers, controlling organ morphology, or being required for the differentiation or function of specific cell types. The role of polyploidy in wound healing and regeneration as well as developmental pathways controlling polyploidy also are summarized.

Tissue envelopes or barriers composed of polyploid cells

There are several examples in which one tissue layer within an organ is composed of polyploid cells, suggesting that an increase in size of these tissues by cell proliferation may be problematic. This has been defined most clearly for the subperineurial glia (SPG) of the Drosophila nervous system (Figure 2A)[20]. These flat cells are surface glia, bounded on all their sides by septate junctions, a form of tight junction. The SPG provide the blood-brain barrier in Drosophila, and this requires intact septate junctions [21–23]. Although the size of the nervous system increases markedly during larval development, the number of SPG cells does not increase. Rather, the SPG cells increase in size by increasing ploidy. In the peripheral nervous system and the ventral cord of the central nervous system SPG cells endocycle; in the brain lobes some SPG endocycle, whereas the majority undergo endomitosis and are multinucleate.

Growth of the SPG by polyploidization was demonstrated to be critical to maintain the blood-brain barrier, as a mechanism to coordinate SPG size with the increasing underlying neuronal mass. Inhibition of DNA replication specifically in the SPG during polyploidization reduces ploidy and cell size, with the consequence that the septate junctions rupture and the blood-brain barrier is no longer functional [20]. Intact septate junctions and the blood-brain barrier can be restored after blocking DNA replication in the SPG either by blocking neuroblast proliferation or forcing growth of the SPG by overexpression of Myc, indicative of coordination of the mass of the two tissue layers. Intriguingly, there appears to be active regulation of SPG ploidy by the neuronal mass, because in a brain tumor with increased numbers of neurons ploidy of the SPG increases to retain the blood-brain barrier [20]. The conclusion from these studies is that growth of the SPG by ploidy permits this glial envelope to enlarge to accommodate growth of the nervous system during development without the disruption of septate junctions that would arise from cytokinesis during cell division.

In addition to their role in the blood-brain barrier, SPG recently have been shown to control neural stem cell proliferation in response to nutritional input [24]. This influence requires gap junctions between the SPG to coordinate calcium oscillations in response to nutritional cues, in turn leading to insulin secretion from the SPG. It has not been tested whether this role of the SPG requires them to be polyploid, but given the requirement for gap junctions and the rupture of intercellular attachments that occurs when polyploidization of the SPG is blocked, it is likely that proper size of the SPG through polyploidization is essential for this control of neuroblast number.

The trophoblast giant cells (TGC) of the placenta provide a barrier between the maternal blood supply and the fetus (Figure 2B) [25]. They achieve a ploidy of up to 512C in rodents via the endocycle [26, 27]. Another cell type of the placenta, the syncytiotrophoblasts, are multinucleate, but they are produced by cell fusion [28]. One possibility is that the large size of the TGC and absence of cytokinesis and cell division ensures retention of a continuous barrier between the maternal and fetal compartments of the placenta. Conditional mutation of the transcription factors E2F7 and E2F8, required for the endocycle in TGCs, reduced ploidy by four fold (64C versus 256C at day E9.5), and mitotic divisions were observed in the TGC layer. Nevertheless, fetal viability was not affected [29]. Thus it is possible that polyploidy of TGC is not essential, although the level of TGC ploidy still present in these mice may be sufficient for function.

Polyploid cells potentially may serve a barrier function in mouse and human skin. In both mammals, there is a basal layer containing proliferative cells that give rise to keratinocytes. As the mitotic divisions from the basal layer push keratinocytes towards the surface of the skin, they differentiate and become polyploid by endomitosis or the endocycle (Figure 2C) [30–32]. The junctions between polyploid keratinocytes have not been analyzed, but the presence of large cells, possibly connected into an envelope, could protect the underlying tissue layers. Alternatively, the large cells may have increased resistance to mechanical tension relative to small diploid cells, or provide an advantage by being able to cover a larger surface area in the skin. If present in keratinocytes, the resistance to radiation-induced

apoptosis observed in other polyploid cell types could facilitate stability upon exposure to UV light, and the lack of cell division could protect against tumor formation.

Polyploid cells controlling organ structure

In contrast to polyploid cells composing a distinct tissue layer within an organ, there are organs with dispersed polyploid cells that influence the structure or shape of the overall organ. Two examples are Arabidopsis sepals (the outer layers of the flower) and leaves. The sepals are composed of interspersed giant, polyploid cells and small diploid cells (Figure 2D). Although the ratio of these two cell types is regulated, the spatial distribution varies between sepals. Polyploidization of the giant cells is downstream of epidermal specification factors and regulated by inhibitors of Cyclin/CDK kinases [33, 34]. Overexpression of the inhibitor KIP RELATED PROTEIN (KRP) causes an increase in giant cells at the expense of diploid cells. Mutation of another cell cycle inhibitor gene, *loss of giant cells from organs* (*lgo*), results in loss of giant cells and an increase in diploid cells. These genes also affect the number of giant cells in leaves. Reporters are available whose expression marks the giant cell versus diploid, small cell fate. Determination of giant cell fate is upstream of polyploidy. By contrast, repression of endocyling in the developing sepals leads to ectopic diploid small cells. Thus endocycling not only increases ploidy but also can block cells from assuming the small cell fate.

Overexpression of KRP and *lgo* mutants reveal a critical role for giant cells in controlling sepal and leaf structure [34]. Mutations in epidermal fate genes that cause a loss of giant cells in the sepals or leaves result in morphological changes in which these structures roll inwards. Overexpression of KRP to increase the number of giant cells causes sepals and leaves to bend outwards. Thus the giant cells affect organ morphology, causing curvature of the sepals and leaves.

In the moth *Manduca sexta* pigmentation patterns on the wing are controlled by the size of the cells that build the scales bearing the pigment colors. A recent study confirms the hypothesis that ploidy of the scale-building cells correlates with their size and thus the size of pigmented areas on the wings [35]. Ploidy of scale-building cells varies by position across the wing, being highest (32–64C) at the distal margin.

A complex pattern of endocyling and cell division is required for proper formation of the rectum of adult Drosophila (Figure 2E). The rectum contains four papillar protrusions that regulate salt and water balance. These are composed of polyploid cells and become organized during pupation. Strikingly, the papillar cells become polyploid during pupation but then undergo two mitotic cell cycles, the only known example of a Drosophila endocycling cell reverting to the archetypal cell cycle [18]. The mitotic divisions of the 8C cells are marked by high chromosome segregation errors. Despite this, analysis of loss of function for the Fizzy-related protein, an activator of the Anaphase Promoting Complex necessary for endocycles, indicates that endocycles are necessary in rectal papillar cells for proper organogenesis of the rectum [36].

Another potential example of polyploid nuclei affecting organ function is the yolk layer in zebrafish embryos. This layer contains syncytial nuclei that become polyploid as

gastrulation proceeds, a frequent property of yolk nuclei [37]. Although the function of increased ploidy within the yolk nuclei has yet to be evaluated, given the role that the yolk appears to play in the morphological movements associated with epiboly and gastrulation, it will be interesting to explore if the size of nuclei influences these events.

Polyploidization linked to the differentiation or function of specific cell types

In addition to the role of polyploid cells in affecting the morphology or function of an organ, polyploidy can be necessary for the function of some cell types. Perhaps the most dramatic example is the neurons of slugs. In Aplysia, the nuclei of the giant neurons, cells 1mm in diameter, are 200,000C [38]. Other slugs have giant neurons that are 10,000C [39]. The neurons of the CNS ganglia also reach enormous levels of ploidy in other, but not all, gastropods (see [40] for a survey). These animals appear to require giant neurons to innervate large regions [40, 41].

Polyploidization of the heart muscle cardiomyocytes serves as a growth control mechanism while avoiding disrupting the sarcomere actin-myosin structure by cell division [42]. Although mammalian cardiomyocytes proliferate during embryogenesis or fetal development, they do not divide in neonates or adults. Nevertheless, cardiomyocytes grow and increase in size in early postnatal development and in response to myocardial stress such as after surgery or myocardial infarction. This hypertrophy is the consequence of endomitosis, not cell fusion as in other muscle cells [43]. Most cardiomyocytes become binucleate tetraploids, but up to 20% of the cells have higher numbers of nuclei [44]. In addition to endomitosis, some mouse cardiomyocytes also endocycle [45]. The sarcomere, critical for contraction, appears to be maintained by increasing cardiomyocyte cell size rather than number. The increased cell size from polyploidization may facilitate cardiac muscle contraction under stress conditions [42].

Mammalian megakaryocytes are giant cells derived from the hematopoietic lineage whose large size is necessary for them to bud off sufficient numbers of platelets from their cytoplasm (Figure 2F). Megakaryocytes reach ploidies of 128C via an unusual form of endomitosis in which they enter mitosis, proceed through to separation of sister chromatids in anaphase A, but then exit mitosis without nuclear division or cytokinesis [46, 47]. Disruption of ploidy of megakaryocytes by reducing the activity of key cell cycle regulators reduces platelet production. A recent study demonstrated that ploidy, and thus cell size, is the critical element for megakaryocyte function rather than endomitosis per se [48]. Ablation of Cdk1 in vivo blocks mitosis in megakaryocyte differentiation, but ploidy increases continue via the endocycle, and adequate platelet formation occurs.

Trichomes are hair-like structures, and in Arabidopsis the leaf trichomes have a characteristic branched morphology dictated by ploidy [49]. The trichome progenitor cells undergo two or three rounds of endocycling prior to morphogenetic elongation and branching. Ploidy affects not only the size of the trichomes but also the number of branches: increased ploidy results in extra branches and reduced ploidy in fewer than the number normally arising in the 32C trichome cells. The trichomes are associated with resistance to insects [50], making it likely that branching is relevant and that ploidy impacts differentiation and function. In addition to affecting trichome morphology, loss of the

endocycle in *sim* mutants (a CDK inhibitor), changes patterning with a reduction of trichome initiation sites [51]. Thus in these cells endocycling may be integrally linked to determination and/or differentiation. Perturbation of endocycle regulation coupled with live imaging confirmed that absence of mitosis is required to maintain trichome cell fate [51].

Polyploidization is critical for cell function of the support cells in Drosophila oogenesis. The oocyte is linked to 15 sister nurse cells by cytoplasmic bridges. While the oocyte enters meiosis, these nurse cells enter the endocycle, attaining ploidy levels of 512–1024C [52]. The genomic copies permit the robust gene expression required for the factory function of the nurse cells to deposit maternal components into the developing oocyte. The cytoplasmic bridges are key for transfer of the nurse cell products into the oocyte, thus a limited number of polyploid nurse cells rather than 500 diploid cells is needed [53]. Mutations that disrupt polyploidization of the nurse cells result in degeneration of the nurse cells and oocyte midway through oogenesis [54].

The somatic support cells for the oocyte, the follicle cells, also become polyploid, although only to 16C [55]. This is needed for production of the eggshell coverings by the follicle cells, as even the 16 copies of major eggshell protein genes are inadequate for the levels of gene expression required in a brief developmental window. The gene copy number must be augmented by amplification of the eggshell protein genes by re-replication of specific genomic regions following the completion of the endocycles (Figure 2G) [56]. It is not clear, however, whether the endocycles are a necessary prerequisite for subsequent chorion gene amplification.

A profound role for polyploidy is the effect of ploidy levels of the hypodermis on the entire nematode body size. The hypodermis is formed during early larval development by cell fusion to generate a multinucleate syncytium. But in *C. elegans* 50% of organismal growth occurs during the adult stage, and this is associated with increased ploidy of the hypodermal nuclei by endocycles. To evaluate potential roles for the hypodermal syncytium in animal size, Flemming et al. compared genome size, nuclear number, and nuclear ploidy in the hypodermis to adult size in 12 nematode species with a 100 fold range in volume [57]. They found no correlation between genome size transmitted through the germline and adult body size, but rather the somatic ploidy of the hyperdermis was tightly linked. This appears to be causal at least in *C. elegans*, as mutations disrupting TGF-β signaling alter hypodermal cell ploidy and body size coincidently [57, 58]. In addition, altering hypodermal ploidy by inhibiting DNA replication or the endocycle resulted in small body size [59]. It is not clear how hypodermal ploidy affects the entire body size. This cell is responsible for producing the body wall muscle and cuticle, so it is possible that the extent of muscle and cuticle synthesized depends on the size of the hypodermis, thus controlling overall body size.

Polyploidy in wound repair and organ regeneration

The above highlights emphasize the role of polyploidization during normal organogenesis in development, but it additionally functions in tissue repair and organ regeneration, as noted above for cardiomyocytes. Wound repair has been examined in the abdominal epidermis of adult Drosophila [60]. Unexpectedly, following wounding the epidermis is repaired by the formation of polyploid mononucleate cells as well as large syncytial cells by cell fusion,

rather than via a restoration of cell number (Figure 2H). The production of both syncytial and polyploid cells at a wound site requires the Yki transcription factor, and the two cell types act redundantly for wound closure. The authors propose that these large cells stabilize the wound site for repair. In another study in Drosophila, it was observed that if endocycling follicle cells undergo apoptosis, surrounding follicle cells compensate to maintain the epithelial layer by undergoing accelerated, additional endocycles, thus increasing their size [61]. Insulin signaling is required for this follicle cell hypertrophy.

Liver hepatocytes become polyploid after weaning in the mouse and in humans, attaining 90% and 50% of the hepatocytes being polyploid in mice and humans, respectively [2, 62]. Hepatocytes can be mono or binucleate, and the ploidy of each nucleus can be 8C [63]. Polyploidy can be induced by oxidative stress, telomere damage, iron overload or partial hepatectomy. Polyploidy in the liver has been proposed to increase metabolic capacity, protect against DNA damage, or provide genetic variation from the multiple gene copies. Regarding the last hypothesis, polyploid hepatocytes are not terminally arrested and are capable of resuming proliferation, frequently with chromosome segregation errors to produce aneuploid daughter cells [2]. A "ploidy conveyor" has been proposed for hepatocytes, in which they can move from being diploid to octaploid but then reverse ploidy (for example during regeneration) back to diploidy, or aneuploidy if segregation errors occur [2].

Direct observation of labeled hepatocytes during regeneration following partial hepatectomy, however, revealed that the first steps involve hypertrophy, most likely via increased ploidy [64]. Thus this study concludes that the initial event in regeneration is increased hepatocyte size rather than number, and proliferation contributes to regeneration only if the majority of the liver is removed. The E2F7 and 8 transcription factors are required for polyploidization of hepatocytes. A confounding observation is that conditional knock out of these genes did not apparently affect hepatocyte differentiation or liver regeneration [65]. This raises the possibility that polyploidization is dispensable for hepatocyte function. It may be required under conditions of severe stress or damage not examined in this study. Direct observation of *E2F7,8* mutant hepatocytes also may uncover defects in regeneration not detected by measuring liver weight.

Developmental regulation of polyploidy

Extensive progress has been made in defining the alterations in cell cycle parameters and regulators responsible for the endocycle and endomitosis. There are several excellent recent reviews detailing control of these variant cell cycles [49, 66–68]. Briefly, E2F transcription factor family members are critical for the endocycle in plants, mammals, and Drosophila. In Drosophila, S-G oscillations result from cyclic synthesis and degradation of E2F1[69], and this is the key driver of the endocycle. In Drosophila, plants and TGCs Cyclin/CDK inhibitors are crucial in the transition from proliferation to the endocycle.

Delineation of the developmental cues responsible for promoting polyploidy has not advanced as far as our understanding of regulation of these variant cell cycles. A few critical roles for developmental regulators have been detailed in the examples of polyploidy discussed above. In Drosophila, transcriptional control of *cyclin E* by developmental

regulatory genes contributes to the developmental patterning of endocycle S phases in embryogenesis [70, 71]. Notch signaling is necessary for the transition from the archetypal cell cycle to the endocycle in the Drosophila adult midgut and follicle cells [56, 72]. Notch also influences rectal papillae development, but by promoting mitosis rather than the endocycle [18]. Epidermal specification genes have been shown to act upstream of the endocycle in Arabidopsis giant cells in the sepal and leaf [34], and the CDK inhibitor SIM is controlled by transcription factors that specify trichomes [73]. Thrombopoietin is the upstream switch for megakaryocyte differentiation that puts the pathway in place for endomitosis [46]. There is a mouse cell line of TGC precursor cells that can be induced to endocycle by the removal of Fibroblast Growth Factor 4 [74]. TGF-β signaling is involved in promoting the endocycle in the nematode hypodermis [57, 58], and insulin-Akt signaling promotes hepatocyte polyploidization [75]. Further research is necessary to determine whether pathways such as Notch, insulin and TGF-β are used universally to control polyploidization and how much diversity exists in distinct developmental contexts.

Concluding remarks

An intriguing set of organs implementing polyploidization as a growth strategy has been identified in plants and animals, raising a set of fascinating questions about how this adaptation arose evolutionarily and how it is mechanistically regulated (Box 1). With increasing awareness of the role of polyploidy in size control more examples of polyploid cells in organs are likely to be uncovered, and these may reveal yet additional uses for this strategy. A current limitation in the field is the uncomfortable extent to which functions for polyploid cells rely on correlative observations or hypotheses. The optimal experiment to test for a functional requirement for polyploidization is to be able to generate an organ of comparable mass composed solely of diploid cells. This has not been possible, given that in most cases polyploidization is downstream of differentiation and the difficulties in returning an endocycling cell to mitosis. But experiments such as the E2F conditional gene deletions offer a means to decipher essential requirements for and functions of polyploid cells. Advances in bioengineering will further our understanding of how polyploid large cells contribute to mechanical strength and tension. The answers to these questions, at the interface between developmental regulation and cell cycle modification, will lay a critical foundation for the frontier of the control of organ size.

Acknowledgments

GLOSSARY

Endocycle	A variant cell cycle with oscillating gap and DNA synthesis phases which produces mononucleate polyploid or polytene cells (Figure 1B)
Polyploid	A multiple, integral increase in the haploid genome content, defined in C values, where C is the haploid genome content. When chromosomes can

be visualized, ploidy can be expressed by N values, where N is the haploid number of chromosomes. Note that for simplicity in this review ploidy levels are reported solely as C values

Polytene   Specialized polyploid cells in which the replicated chromatid copies remain physically attached. For example, Drosophila salivary gland cells are 1024C but 1N

Endomitosis   Here the definition is broadened to include several variant cell cycles in which aspects of mitosis occur, such as nuclear envelope breakdown, anaphase, and/or nuclear division, but not cytokinesis (Figure 1C)

Differential Replication   In many polyploid cells the genome is not integrally replicated, with regions repressed for replication and thus reduced in copy number or overreplicated and thus amplified in copy number

Aneuploid   A cell with reduced or increased numbers of a single or subset of chromosomes, as opposed to the integral increases in the complete chromosome set observed in polyploid cells

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OUTSTANDING QUESTIONS

1. It is of crucial importance to provide functional evidence for a requirement for polyploidy as a growth mechanism in organogenesis.

2. Experiments are needed to determine whether polyploid cells have higher metabolic activity and whether increased ploidy augments gene expression.

3. How does the hypertrophy resulting from polyploidization affect the mechanical properties of cells? What are the properties of a tissue layer composed of large polyploid cells compared to smaller diploid cells in terms of resistance to mechanical tension and force transduction?

4. Is the absence of apoptosis in polyploid cells a physiological advantage?

5. Do multiple versus single nuclei affect polyploid cell function, or is total ploidy of sole importance?

6. How prevalent is differential DNA replication in polyploid cells?

7. How is total organ mass monitored and the mass of individual tissues coordinated?

8. How do developmental regulators impact the cell cycle to transition between archetypal and variant cell cycles?
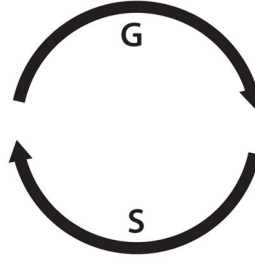
Highlights

- Large cells required in plant and animal organs are generated by increased ploidy

- A large cell has multiple advantages versus a comparable mass of diploid cells

- Cell hypertrophy via increased ploidy can produce an envelope or control organ shape

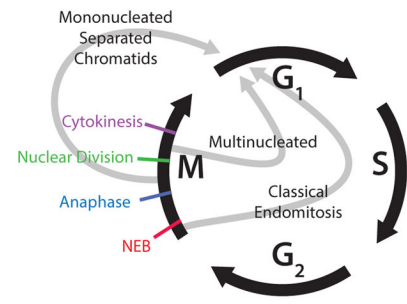- Polyploidy is linked to cell differentiation and function
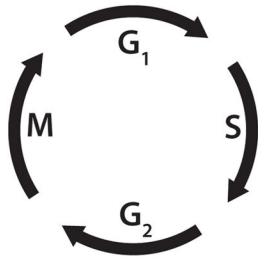
(A) Archetypal Cell Cycle   **(B) Endocycle**   **(C) Endomitosis**

Figure 1.

Cell Cycle Variants Yielding Polyploid Cells. (A) The archetypal cell cycle responsible for cell proliferation contains a G1 phase during which sufficient cell growth must occur prior to the onset of DNA replication in S phase. Another gap phase (G2) precedes mitosis and the return to G1 in the two daughter cells. (B) The endocycle involves oscillations between a gap phase and S phase and can produce either polyploid or polytene cells, distinguished by whether the replicated chromatids remain in physical association. (C) Endomitosis is distinguished by entry into mitosis but a failure to complete all aspects of mitosis. This can involve a failure of nuclear envelope breakdown but assembly of a spindle within the nucleus and segregation of sister chromatids, nuclear envelope breakdown and anaphase segregation, or completion of all of the events of mitosis, including nuclear division, but without cytokinesis.
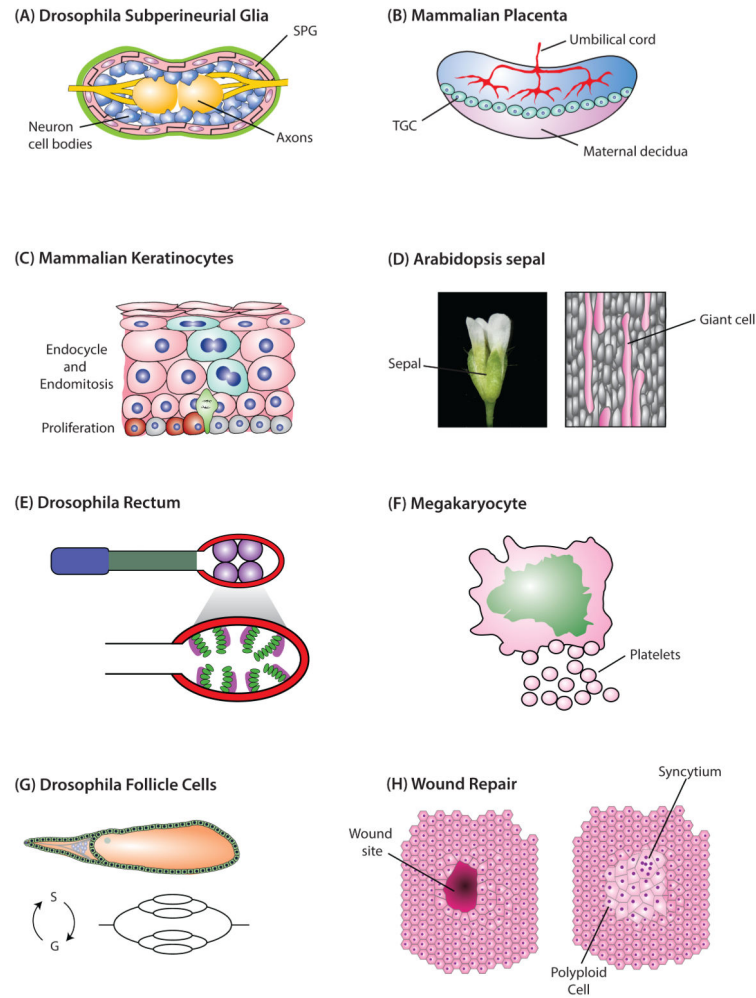
Figure 2.
Examples of Polyploid Tissues within Organs. (A) The subperineurial glia (SPG, pink) are surface glia in the Drosophila nervous system, surrounding the neuronal cell bodies (blue) and axons (yellow). Increased size of the SPG resulting from polyploidy is required to maintain the blood-brain barrier as neuronal mass increases during development. (B) The trophoblast giant cells (TGC, light blue) make a barrier between the maternal and fetal compartments of the placenta. (C) In mouse and human skin, there is a basal layer with quiescent (grey) or proliferating cells (red). Cell division (green cell) moves daughter cells towards the outer layer of the skin (top of drawing). In the suprabasal layers the keratinocytes undergo endomitosis (blue) and the endocycle (pink) to increase their ploidy and size. (D) In the sepal, the outer layer, of Arabidopsis flowers (left) there are both giant, polyploid cells (pink cells, right) and diploid cells (grey, right). The numbers but not positions of the two cell types are regulated. (E) The Drosophila rectum contains four papillae composed of polyploid cells that endocycle for two divisions then divide to produce the required number of polyploid cells in each papillae. The bottom figure represents an enlargement of the rectal papillae, with the 8C cells that underwent endocycles followed by mitotic divisions shown in green and Delta expressing cells shown in purple. (F)

Megakaryocytes have a nucleus (green) with up to 128C DNA content with multiple invaginations in the nuclear envelope. The large cell size resulting from polyploidy is required for the megakaryocytes to bud off sufficient numbers of platelets (small pink circles). (G) The Drosophila ovarian follicle cells (green) endocycle to 16C (bottom left). Genomic replication is then shut off, but six genomic regions undergo repeated origin firing and gene amplification (bottom right). (H) In wound healing in the Drosophila epidermis polyploid cells that are either in a multinucleate syncytium or mononucleate cells are produced at the wound site.

Table 1

Cell Types Discussed

Cell Type	Organ	Organism	Maximum Ploidy	Cell Cycle	Function of Increased Ploidy	Ref
FoI, Eisen Cells	Epithelium	O. dioica	1300C	Endocycle	Control body size and nutritive input?	10
Subperineurial Glia (SPG)	Nervous System	Drosophila	32C	Endocycle and Endomitosis	Increased cell size to maintain blood-brain barrier	20
Trophoblast Giant Cell (TGC)	Placenta	Rodents	512C	Endocycle	Increased cell size for placental barrier?	26–29
Keratinocytes	Skin	Mouse Human	12C	Endocycle and Endomitosis	Increased cell size for protective function of skin?	30–32
Giant Cells	Leaf and Sepal	Arabidopsis	16C	Endocycle	Controls curvature of leaves and sepals	33, 34
Scale-building cells	Wing	Manduca	16C–64C	Endocycle	Determines cell size and thus pigmented regions	35
Rectal Papillae	Intestine	Drosophila	8C	Endocycle	Endocycle followed by mitotic divisions necessary for papillae formation and control of salt and water absorption	18, 36
Syncytial Yolk Nuclei	Embryo	Zebrafish	8C–40C	? Endocycle	? Function	37
Giant Neuron	Nervous System	Aplysia	200,000C	? Endocycle	Sufficient neuronal size to innervate large area	38
Giant Neuron	Nervous System	Limax Slug	10,000C	? Endocycle	Sufficient neuronal size to innervate large area	39
Cardiomyocytes	Heart	Mouse	4C–8C	Endomitosis Endocycle	Mechanism for cell growth postnatally and in response to cardiac damage	43–45
Megakaryocyte	Blood	Mammals	128C	Endomitosis	Large cell size required for sufficient platelet production	46–48
Trichomes	Leaf	Arabidopsis	32C	Endocycle	Controls formation and branching in trichomes	49, 51
Nurse cells	Ovary	Drosophila	512C	Endocycle	Synthesis and transport maternal stockpiles to oocyte	52
Follicle cells	Ovary	Drosophila	16C	Endocycle	Synthesis of eggshell	55
Hypodermal cell (Hyp7)	Hypodermis	C. elegans nematodes	12C	Endocycle	Endocycling to increase ploidy following cell fusion. Ploidy levels control body size	57–59
Hepatocytes	Liver	Mammals	16C	Endomitosis Endocycle	First observed step in regeneration	2, 62–65