Review

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When bigger is better: the role of polyploidy in organogenesis

Terry L. Orr-Weaver

Whitehead Institute and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142, USA

Defining how organ size is regulated, a process controlled not only by the number of cells but also by 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 widespread 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 the proper arrangement of these cell types into tissues. A second crucial but poorly understood aspect of organogenesis is the 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 the regulation of cell proliferation. I discuss here 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, except in some plants cells in which increase in vacuole size expands the cell [6]. Nuclear volume and total cell size scale with DNA content, and the polyploidy present in large cells indicates that there is a minimal ratio of nucleus to cytoplasm throughout biology [7]. A complete understanding of organ and tissue size therefore requires delineating the regulation of cell polyploidization in development as well as its coordination with cell proliferation and differentiation.

Overview of somatic polyploidy

The role of polyploidy in development is distinct from species ploidy because 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; see Glossary). The endocycle contains solely a gap (G) phase, during which gene expression and growth take place, and a DNA synthesis (S) phase [8]. In the endocycle all aspects of mitosis are shut off. The endocycle

Glossary

Endocycle: a variant cell cycle with oscillating gap (G) and DNA synthesis (S) phases that 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, and some regions are repressed for replication, and are thus reduced in copy number, or are over-replicated 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.

Corresponding author: Orr-Weaver, T.L. (weaver@wi.mit.edu).
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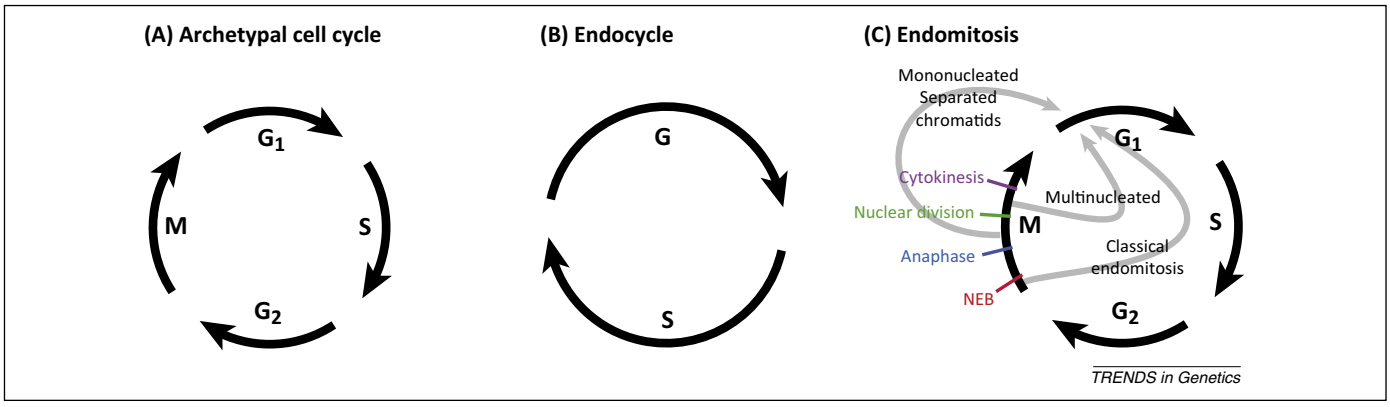

Figure 1. Cell cycle variants yielding polyploid cells. **(A)** The archetypal cell cycle responsible for cell proliferation contains a G1 phase during which sufficient cell growth must occur before the onset of DNA replication in S phase. Another gap phase (G2) precedes mitosis and the return to G1 in the two daughter cells. **(B)** The endocycle involves oscillations between a G phase and S phase, and can produce either polyploid or polytene cells, differing by whether the replicated chromatids remain in physical association. **(C)** Endomitosis is distinguished by entry into mitosis but failure to complete all aspects of mitosis. This can involve 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. Abbreviation: NEB, nuclear envelope breakdown.

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*, because 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: (i) large cells can act as a tissue envelope; (ii) the absence of mitosis and cell division has been argued to permit polyploid cells to be more metabolically active [13]; (iii) 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, because they can acquire different characteristics as they divide, whereas the polyploid cell can have a more stable, differentiated state; (iv) multiple genome copies within a cell provide protection against mutations and thus confer damage resistance; and (v) apoptosis is inactivated in at least some polyploid cells, possibly lengthening lifespan [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].

Below 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 crucial for tissue layers, controlling organ morphology, or being required for the differentiation or function of specific cell types. The roles of polyploidy in wound healing and regeneration, as well as developmental pathways controlling polyploidy, are also 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