cats often have a blue eye ipsilateral to the deaf ear and a non-blue eye ipsilateral to the hearing ear. To eliminate this inherent variability, hearing screening must be performed early in life. If diagnosed as deaf in this screening, the resulting animals are called 'congenitally deaf cats'. Even non-white cats can theoretically be deaf, but the proportion of such animals is very low.

Do deaf white cats have any other functional deficits? Theoretically, genes causing deafness may affect other bodily functions. However, white cats, even congenitally deaf cats, behave otherwise normally. Several studies directly compared parts of the brain and its functions to hearing animals. Neither in the somatosensory, motor nor the visual system are congenitally deaf cats impaired compared to hearing animals. In fact, it has been demonstrated that the visual functions of congenitally deaf cats are supranormal, presumably to compensate for the loss of hearing, and they have enhanced integrative visual functions like visual motion detection and localization. For these supranormal functions, portions of the acoustically-deprived auditory cortex are recruited. Similar supranormal visual functions have been observed in perinatally deaf humans.

Are deaf white cats different from deafened animals? In addition to hereditary deafness, hearing loss can also be induced by local or systemic application of drugs that destroy hair cells. The histology of the inner ear differs between deafened animals and deaf white cats. Ototoxic drugs as a rule destroy not only hair cells but also other cells in the organ of Corti and neurons that give rise to fibers of the auditory nerve. In combination, this leads to pronounced loss of auditory nerve fibers that provides an additional complication when comparing hearing and deafened animals. However, the advantage of pharmacological deafening is that it can be induced at any age. Consequently, to understand the complex effects of deafness, it needs to be investigated in both congenital and acquired deafness models.

Can hearing be restored in deaf white cats? Yes, it can. In fact, deaf cats have been an exceptionally useful model for studying effects of such restoration in the brain. The feline auditory system is similar to humans; cats can perform similar acoustic functions as humans. Their brain is gyrencephalic (unlike rodents), containing many cortical auditory areas (over a dozen), and cats have a similar low-frequency hearing ability to humans (which is not the case, for example, in mice or rats). Furthermore, cats are an excellent model for investigating multimodal interactions, as cats are highly visual and auditory, unlike rodents that mainly use their hearing and their somatosensory system (whiskers) for orientation. As the cochlea of these animals is large enough, a neuroprosthetic device, called a cochlear implant, can be used to restore hearing at nearly any age. Such studies have revealed that early hearing is important for normal development and maturation of the auditory system. Furthermore, limits of such plasticity ('sensitive periods') and their possible mechanisms have been explored using this animal model.

Where can I learn more?

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Primer

Polyploidy

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and Terry L. Orr-Weaver

Polyploidy is defined as an increase in genome DNA content. Throughout the plant and animal kingdoms specific cell types become polyploid as part of their differentiation programs. When this occurs in subsets of tissues within an organism it is termed somatic polyploidy, because it is distinct from the increase in ploidy that is inherited through the germline and present in every cell type of the organism. Germline polyploidy is common in plants and occurs in some animals, such as amphibians, but will not be discussed further here. Somatic polyploid cells can be mononucleate or multinucleate, and the replicated sister chromatids can remain attached and aligned, producing polytene chromosomes, or they can be dispersed (Figure 1). In this Primer, we focus on why somatic polyploidy occurs and how cells become polyploid — the first of these issues being more speculative, given the status of the field.

Why cells become polyploid

The clearest general use of somatic polyploidy appears to be as a mechanism to produce large cells. This is exploited in some developmental contexts in which fewer, larger cells have functional advantages over a similar total mass of an increased number of smaller cells. Polyploidy additionally may augment gene expression or metabolism.

Polyploidy as a means to increase cell size

One use of polyploidy is to generate large cells, such as mammalian megakaryocytes or the giant cells that contribute to the structure of organs such as *Arabidopsis* leaves. It has been appreciated since late in the 19th century that cell size is proportional to nuclear size, and this was subsequently shown to reflect DNA content. Thus, both polyploid and polytene cells are of increased size, some attaining sizes orders of magnitude larger than diploid cells, with corresponding

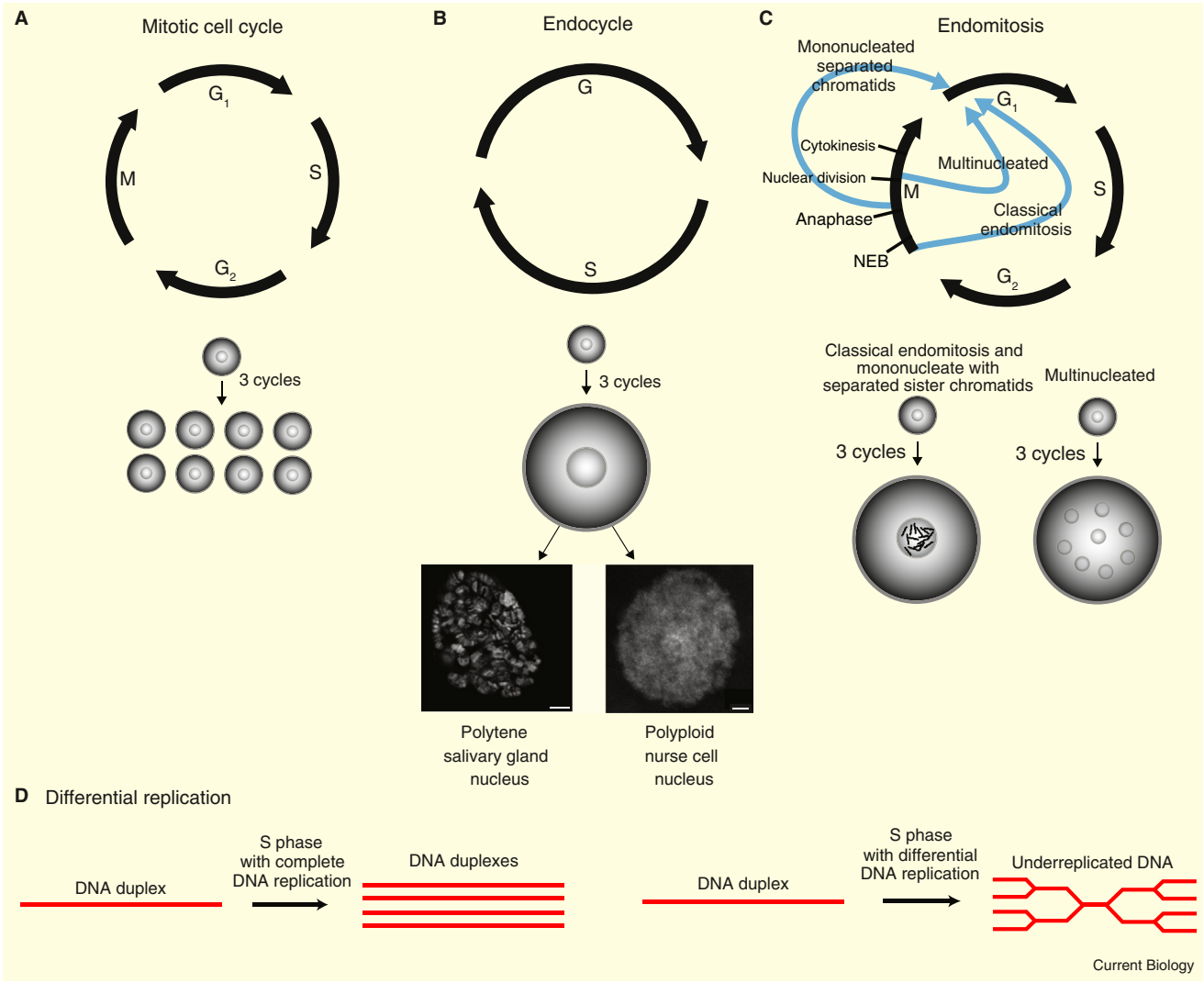

Figure 1. Cell cycles producing polyploid cells.
(A) The mitotic cycle contains four distinct phases that produce two identical daughter cells. G1 and G2 are growth phases during which gene expression and growth occur, DNA replication takes place during S phase, and mitosis occurs during M phase. (B) The endocycle is driven by repeated S–G cycles that lead to polyploid or polytene cells. A distinct banding pattern can be seen in the image of *Drosophila* polytene salivary glands, which can reach 2048C, where C is the haploid genome content for the organism. This banding pattern is reflective of the replicated sister chromatids being tightly aligned. Scale bar, 5 μm (image from Jessica Von Stetina, Whitehead Institute). In contrast, nurse cells have no distinct banding pattern in late oogenesis and can reach 512C. Scale bar, 20 μm (image from Boryana Petrova, Whitehead Institute). (C) Endomitotic cells enter mitosis, but differ in which mitotic steps they complete. Endomitosis was initially defined as a process in which a spindle assembled to segregate sister chromatids within an intact nucleus (classical endomitosis). Additional variants now also are termed endomitosis, such as in cell types in which nuclear envelope breakdown and some aspects of chromosome segregation occur but nuclear division does not. This, like classical endomitosis, produces mononucleate cells. Completion of mitotic events through nuclear division in the absence of cytokinesis leads to multinucleate endomitotic cells. (D) During polyploidization, integral doublings of the genome do not necessarily occur. As schematized here, repression of replication can lead to underreplication and reduced copy number of specific regions relative to overall ploidy. Less commonly, overreplication can produce domains of amplified gene copy number (not shown).

increases in genomic DNA (Figure 2). Although increased DNA content is consistently associated with increased cell size, there is not an absolute ratio between nuclear size and cell size (the karyoplasmic ratio) across cell types. Cell-size increases associated with increased DNA content can be dramatic, producing cells visible to the naked eye, such as giant neurons in slugs (diameter of 1 mm) and the polyploid trichome cells of *Arabidopsis* (1 mm in length). Even in bacteria, cell size is coordinated with DNA content.

The correlation between increased DNA content and very large cell size suggests that there is a requirement for a minimal nuclear to cytoplasmic ratio and a limitation to how large cells can become by increasing mass through growth alone. One example cited as an argument against a need for increased DNA content with a large cell size are eggs, which can fill with yolk to attain

an enormous size. In 1912 Conklin tested which components of cytoplasm could reflect nuclear size, evaluating the contribution of yolk by separating it from cytoplasm and measuring cell size during early cleavage in sea snail embryos. He concluded that the relationship between nuclear size and cell size was determined not by the total cytoplasm but by the active cytoplasm without yolk.

Another example that would seem to argue against a minimal nuclear to cytoplasmic ratio is neurons: these cells can be extremely large as a result of their axons, which can be a meter or more in length. A barrier excludes cytoplasm from the axons, however, making it likely that axonal volume is not monitored as part of the cytoplasmic volume. Purkinje cells are neurons that can be enormous due to large dendritic arbors, raising the question of their nuclear to cytoplasmic ratio. There are conflicting reports in the literature regarding whether or not these cells are polyploid, and reinvestigation of the ploidy of Purkinje cells with newer microscopy tools would be highly informative.

We still do not know which components contribute to the size relationship between the nucleus and the cell or why increased DNA content leads to an increase in cell size. Although it had been proposed that causality perhaps occurs in the reverse direction, with increased cytoplasmic volume leading to increased nuclear size, *Drosophila* and plant mutants in which DNA content is primarily affected clearly establish that cell volume follows nuclear size. One possibility that will be valuable to investigate is whether the gene copy numbers of components of the ribosome (ribosomal proteins or rRNA) are limiting, making cell size above a threshold dependent on increased ploidy. Such a possibility could also explain why increased ploidy leads to increased cell size, if it results in increased numbers of ribosomes that lead to increased translation and cellular mass.

A potential complication of the use of ploidy as a means to increase cell size is that, for a sphere, surface area increases with the square of the radius whereas volume increases with the cube of the radius. Thus, with increasing DNA content the nuclear surface area will not keep up with increasing nuclear volume: if the nuclei are flat, however, the two parameters will scale together. It is notable that in many polyploid cell types in both plants and animals the nuclei are flat rather than spherical and in addition contain many involutions in the nuclear envelope. We speculate that these may be mechanisms to increase the surface area of the nuclei: adequate surface area could be critical for sufficient numbers of nuclear pores to ensure adequate mRNA transport from the nucleus.

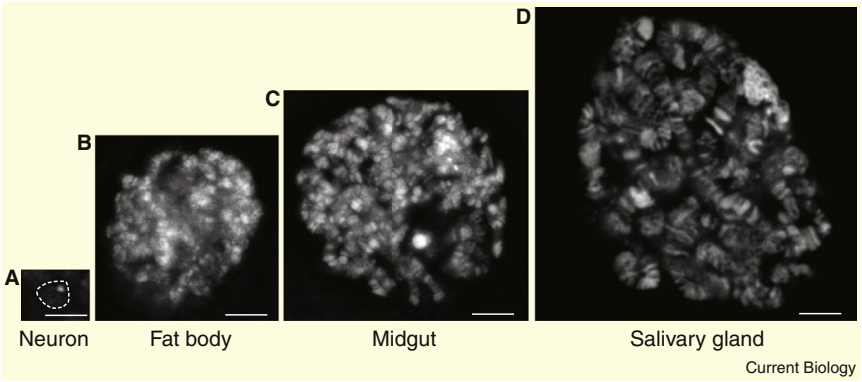

Figure 2. Correlation of nuclear size with DNA content.
DAPI-stained nuclei isolated from various *Drosophila* tissues: (A) third-instar larval brains, (B) fat body, (C) midgut, and (D) salivary glands are shown (all shown to the same scale with scale bars of 5 μm). A single diploid neuronal nucleus is outlined in (A). The ploidy level quantified for the fat body nucleus shown is 74C, the midgut 177C, and the salivary gland nucleus is 1824C. Although the mean ploidy levels for fat body nuclei are 225C, 171C for midgut, and 1669C for salivary gland, there is a range of nuclear ploidy within each of these tissues. (The image in (A) is from Laura Frawley, (B,C) from Sharon Li, and (D) from Jessica Von Stetina, all at the Whitehead Institute.)

Specialized functions of polyploid cells

Growth by polyploidization rather than by an increase in cell number via proliferation provides advantages in some developmental contexts. In organs in which one tissue layer provides a barrier, such as the glial tissue layer that makes the blood–brain barrier in *Drosophila*, the trophoblast giant cells (TGCs) that separate maternal and fetal compartments of the placenta, and possibly the keratinocytes in mammalian skin, cell division may disrupt barrier function. Growth by polyploidization enables cells to maintain intercellular junctional integrity while increasing in size by orders of magnitude to accommodate an increasing mass of underlying tissue layers in development. Megakaryocytes need to be large in size to fulfill their function in platelet production, which occurs via platelet budding from the cytoplasm. Polyploidy is necessary to produce megakaryocytes with an adequate cytoplasmic volume to produce sufficient numbers of platelets.

Polyploidy as a strategy for increased gene expression or metabolism

Some polyploid cell types are highly metabolically active, such as *Drosophila* nurse cells, which synthesize and provide the oocyte with mRNAs, proteins, translational machinery and mitochondria. Thus, another advantage of polyploidy could be that increased gene copy numbers facilitate high rates of biosynthesis and metabolism. This currently remains a hypothesis, however, until studies are carried out to examine whether gene expression levels are increased in polyploid cells, i.e. whether the levels of transcript per gene per polyploid cell are higher than those in diploid counterparts. Recent techniques to rigorously quantify transcript levels per cell using normalization of RNA sequencing now permit the extent of the effect of polyploidy on gene expression to be analyzed in a large number of cell types. In addition to investigating gene expression, evaluation of the function of polyploidization will benefit from comparisons of translation and metabolic rates in polyploid versus diploid cells.

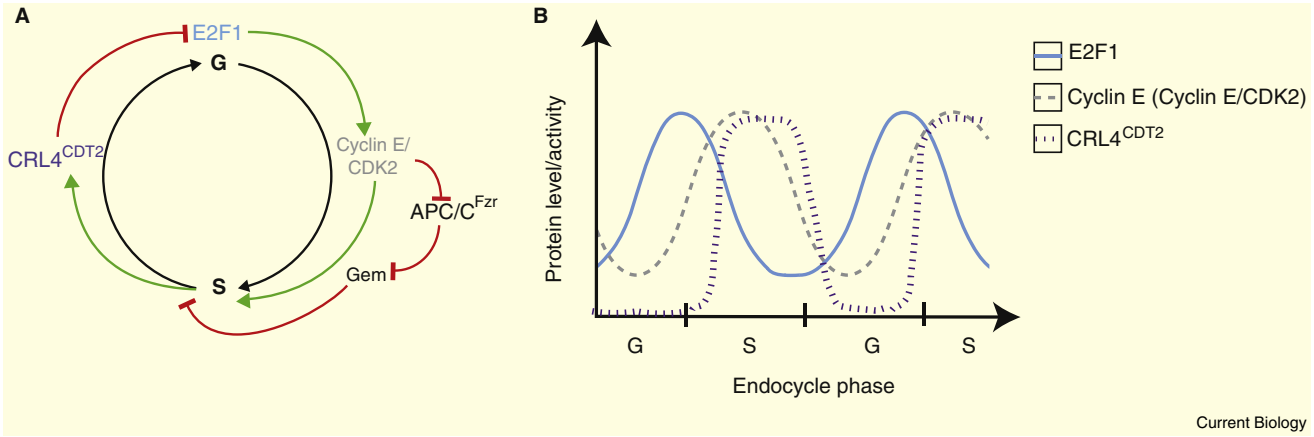

Figure 3. Endocycle progression in *Drosophila*.
(A) Regulatory inputs to the endocycle. The E2F1 transcription factor promotes expression of *cyclin E*, leading to active cyclin E–CDK2 kinase, which triggers the onset of S phase. Cyclin E–CDK2 inhibits APC/$C^{Fzr/Cdh1}$ activity, thus allowing geminin accumulation and preventing re-replication in a given S phase. Low cyclin–CDK activity and G phase results as $CRL4^{CDT2}$ targets E2F1 for degradation during S phase, leading to a decrease in cyclin E. (B) Oscillation of regulators in the endocycle. Protein levels are indicated for E2F1 and cyclin E and activity for $CRL4^{CDT2}$. Increased levels of E2F1 protein promote increases in cyclin E and thus cyclin E–CDK2 activity. Activation of $CRL4^{CDT2}$ in S phase causes the destruction of E2F1. (Adapted from Edgar *et al*. 2014.)

How cells become polyploid

Polyploidy can be achieved by inhibition of some or all aspects of mitosis in variant cell cycles termed endocycles and endomitosis (Figure 1).

Endocycle onset

Progression through the archetypal cell cycle is dependent on a family of serine/threonine protein kinases known as cyclin-dependent kinases (CDKs). Substrate phosphorylation by specific CDKs enables mitotic cells to progress through G1, S, G2, and M phases of the cell cycle (Figure 1A). However, unlike mitotic cells, endocycling cells lack mitosis and alternate between S and G phases (Figure 1B). To achieve this, endocycling cells must suppress the mitotic machinery, specifically mitotic CDKs, both at the level of transcription of cyclin genes and by affecting cyclin–CDK activity. Transcriptional repression of cyclin genes is not well understood but two mechanisms act on cyclin proteins: proteolytic targeting of mitotic cyclins by the E3 ubiquitin ligase anaphase-promoting complex/cyclosome (APC/C); and inhibition of the activity of cyclin–CDK complexes by CDK inhibitors (CKIs). *Drosophila* cells mainly regulate mitotic CDK proteins via APC/C activation, whereas plants and some mammalian cells rely heavily on CKI activation. Much of our knowledge of polyploidy onset is derived from endocycling cells: the examples detailed below all occur in endocycling cells.

The molecular mechanism of the transition of *Drosophila* mitotic cells into the endocycle has been elucidated in the adult tissues of ovarian follicle cells and the midgut as well as in the developing larval tissues in the embryo. Notch pathway activation in the epithelial follicle cells surrounding the developing oocyte is triggered by the Notch ligand Delta expressed on the underlying germline nurse cells. This leads to inhibition of the Cdc25-type phosphatase String, which normally activates CDKs, and activation of the transcription factor Hindsight (Hnt). Hnt, in turn, prevents the transcriptional repressor Cut from suppressing the APC/C activator, Fizzy-related (Fzr/Cdh1), which leads to the degradation of the mitotic cyclins A, B, and B3. Notch signaling also promotes endocycle onset in enterocytes of the *Drosophila* gut, although the mechanism is not well understood. In contrast to the role of Notch signaling in the ovarian follicle cells, the onset of the endocycle in larval tissues of the *Drosophila* embryo is controlled by expression of APC/$C^{Fzr/Cdh1}$. In *fzr* mutant embryos, epidermal cells undergo an additional round of division, and mitotic cyclins accumulate in post-mitotic salivary gland cells, preventing them from transitioning into the endocycle. Thus, multiple mechanisms can promote the transition into the endocycle in a tissue-specific manner.

CKI activation inhibits CDKs and thus suppresses the mitotic machinery in TGCs. Experiments performed in cell culture demonstrate that, in the presence of fibroblast growth factor 4 (FGF4), the CKIs $p21^{CIP1}$ and $p57^{KIP2}$ are targeted for degradation after being phosphorylated by checkpoint kinase 1 (CHK1), causing trophoblast stem cells to remain in the mitotic cell cycle. Upon removal of FGF4, CHK1 is degraded, allowing $p21^{CIP1}$ and $p57^{KIP2}$ to accumulate and inhibit CDK1 activity, triggering endocycle entry and differentiation into TGCs. Additionally, E2F transcriptional activator and repressor proteins are thought to be involved in the transition into the endocycle, as they affect the expression of mitotic regulators.

Endocycling *Arabidopsis* cells rely on the activity of two plant-specific families of CKIs, SIAMESE (SIM) and SIAMESE-RELATED. SIM binds to the mitotic CDK CDKB1;1 and is also thought to inhibit activation of MYB3R1, a transcription factor that regulates the mitotic cyclin CYCLIN B1. Additional pathways contributing to the suppression of mitotic CDKs include the plant homolog of Fzr/Cdh1, *CCS52A*, E2F–RB complexes, and a second E3 ubiquitin ligase containing CULLIN 4. A number of environmental and hormonal cues also affect the onset of the plant endocycle, as reviewed in Breuer *et al*. (2014).

Cycling

The details of maintenance of the endocycle are better understood than endocycle onset. Repeated G and S oscillations are required to drive endocycles. Specifically, a period of low CDK activity during G phase is required for pre-replication complex (pre-RC) formation, a prerequisite for the initiation of DNA replication in S phase. Mitotic cyclins remain suppressed throughout the endocycle, so only S-phase CDK complexes have to be downregulated. This is achieved by APC/$C^{Fzr/Cdh1}$-targeted degradation of cyclin–CDK complexes and geminin, a protein that inhibits replication licensing. This period of low CDK activity is followed by a period of high CDK activity, which promotes DNA synthesis while simultaneously inhibiting pre-RC formation and origin refiring.

The transcriptional activator E2F1 is a core oscillator essential for endocycle progression. Analyses using *Drosophila* salivary glands clearly demonstrate a biphasic oscillation in which E2F1 accumulates in late G phase and promotes the transcription of *cyclin E*, which leads to a complex of the cyclin E protein with the S phase CDK2 that promotes DNA replication. The S-phase-specific E3 ubiquitin ligase $CRL4^{CDT2}$ targets E2F1 for proteolytic destruction, thereby lowering cyclin E transcription, and re-establishing a period of low CDK activity required for G phase pre-RC assembly. APC/$C^{Fzr/Cdh1}$ contributes to endocycle oscillations by targeting geminin and mitotic cyclins for degradation to allow pre-RC assembly (Figure 3).

E2F also plays a critical role in the establishment of the biphasic oscillator in endocycling mouse TGCs. As in *Drosophila*, E2F transcriptional activators accumulate during late G phase, resulting in activity of the S-phase CDK complex cyclin E–CDK2 during the G-to-S transition. High levels of cyclin E–CDK2 promote DNA replication and inhibit APC/C^{Cdh1} activity, allowing the S-phase cyclin A to accumulate. The cyclin A–CDK2 complex creates a negative feedback loop by phosphorylating cyclin E, thereby targeting it for degradation by the E3 ubiquitin ligase $CRL1^{FBW7}$ (F-box and WD40 domain-containing protein 7). CKIs additionally contribute to TGC endocycle progression, as cyclin E–CDK2 activity is kept low during G phase by $p57^{KIP2}$.

There is some evidence in *Arabidopsis* that oscillations between high and low levels of CDKs contribute to endocycle progression. Both E2F and a second family of CKIs, KIP-related proteins (KRPs; related to mammalian p57 and p21), have been proposed to be involved in what is speculated to be a 'quadruple negative feedback' mechanism. The hypothesized model includes inhibition of the E3 ubiquitin ligase SKP2–Cullin 1–F-box like 17 (SCF^{FBL17}) by RB-RELATED PROTEIN (RBR1), degradation of KRPs by SCF^{FBL17}, inhibition of the A-type CDK CDKA by KRPs, and inhibition of RBR1 via phosphorylation by CDKA.

Endocycling versus endomitotic cells

In contrast to endocycling cells, which suppress mitotic players and oscillate only between S and G phases, endomitotic cells undergo aspects of mitosis and therefore must maintain the expression of some mitotic genes (Figure 1C). Consequently, endomitotic cells need to cycle between G1, S, G2, and aspects of M phase. Trakala *et al*. have recently provided insights into the nature of endomitotic progression in megakaryocytes. The authors addressed which mitotic components are required for megakaryocyte polyploidization and function by ablating a number of mitotic factors, including: CDK1 and/or CDK2 to assess mitotic entry; Aurora B to assess mitotic progression; and the APC/C activator Cdc20 to assess mitotic exit. Interestingly, only ablation of APC/C^{Cdc20} caused mitotic arrest of megakaryocytes, whereas the absence of Aurora B, CDK1, and CDK2 did not significantly affect megakaryocyte polyploidization or platelet levels. Upon CDK1 ablation, however, megakaryocytes switched to undergo polyploidy via the endocycle instead of endomitosis. This switch in polyploidization mechanism is the first evidence that shows that the endocycle can subsititute for endomitosis in megakaryocytes with no obvious detrimental effect on function.

Differential DNA replication

An interesting feature of some endocycling cells is that DNA replication during S phase does not involve an integral doubling of the genome. This differential DNA replication can involve inhibition of replication of specific genomic regions causing reduced copy number, or underreplication (Figure 1D). The opposite has been observed in a limited number of cell types, where overreplication of specific genomic intervals leads to amplification of gene copy number of those regions relative to overall cell ploidy. The latter mechanism is used in a few insect cell types as a means to generate additional templates for robust gene expression. These developmentally controlled amplicons have provided powerful models to decipher the regulation of metazoan replication origin activation and fork progression.

The biological logic for underreplication is less clear. In *Drosophila*, the heterochromatin, which constitutes nearly 30% of the genome, is underreplicated in all polyploid or polytene tissues examined. It may be advantageous to these cells not to invest in duplicating genome regions that are gene poor. Euchromatic regions in *Drosophila* also can be underreplicated, to a lesser extent, with tissue specificity. Underreplication of euchromatic regions is not causally linked to repression of gene expression, and it does not appear essential for viability or differentiation of these polytene cells. Repression of replication at both the heterochromatic and euchromatic regions requires SUUR, a protein that localizes to and inhibits progression of replication forks. Endocycling cells in plants appear to replicate their genomes fully, as do mammalian megakaryocytes. In TGCs the heterochromatin is not underreplicated, but some euchromatic regions of the genome exhibit low, but reproducible, levels of underreplication, usually less than twofold. This indicates either that a subpopulation of the cells undergoes significant underreplication or that in rare S phases some intervals are prone to replication failure.

Future directions

Recent research has focused on a longstanding issue in biology, producing a new appreciation of the strategy of implementing polyploidization.

Systematic examination of the ploidy of differentiated tissues across the plant and animal kingdoms is likely to uncover additional examples and functions for which increased ploidy provides an advantage, as well as potential limitations. In addition to these developmental insights, the field is now poised with powerful new tools to answer key mechanistic questions, such as why does increased ploidy cause an increase in cell size? Is there a minimal karyoplasmic ratio and, if so, why? How are transitions into the endocycle or endomitosis controlled in different developmental contexts? And what are the mechanisms and roles for differential DNA replication? It will be exciting to watch the answers to these questions emerge in different organisms.

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Immediate susceptibility to visual illusions after sight onset

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The dominant accounts of many visual illusions are based on experience-driven development of sensitivity to certain visual cues. According to such accounts, learned associations between observed two-dimensional cues (say, converging lines) and the real three-dimensional structures they represent (a surface receding in depth) render us susceptible to misperceiving some images that are cleverly contrived to contain those two-dimensional cues. While this explanation appears reasonable, it lacks direct experimental validation. To contrast it with an account that dispenses with the need for visual experience, it is necessary to determine whether susceptibility to the illusion is present immediately after birth; however, eliciting reliable responses from newborns is fraught with operational difficulties, and studies with older infants are incapable of resolving this issue. Our work with children who gain sight after extended early-onset blindness, as part of Project Prakash, provides a potential way forward. We report here that the newly sighted children, ranging in age from 8 through 16 years, exhibit susceptibility to two well-known geometrical visual illusions, Ponzo [1] and Müller-Lyer [2], immediately after the onset of sight. This finding has implications not only for the likely explanations of these illusions, but more generally, for the nature-nurture argument as it relates to some key aspects of visual processing.

In the Ponzo illusion (Figure 1A, left), first demonstrated over a century ago, two identical stripes, placed on a background of converging lines,

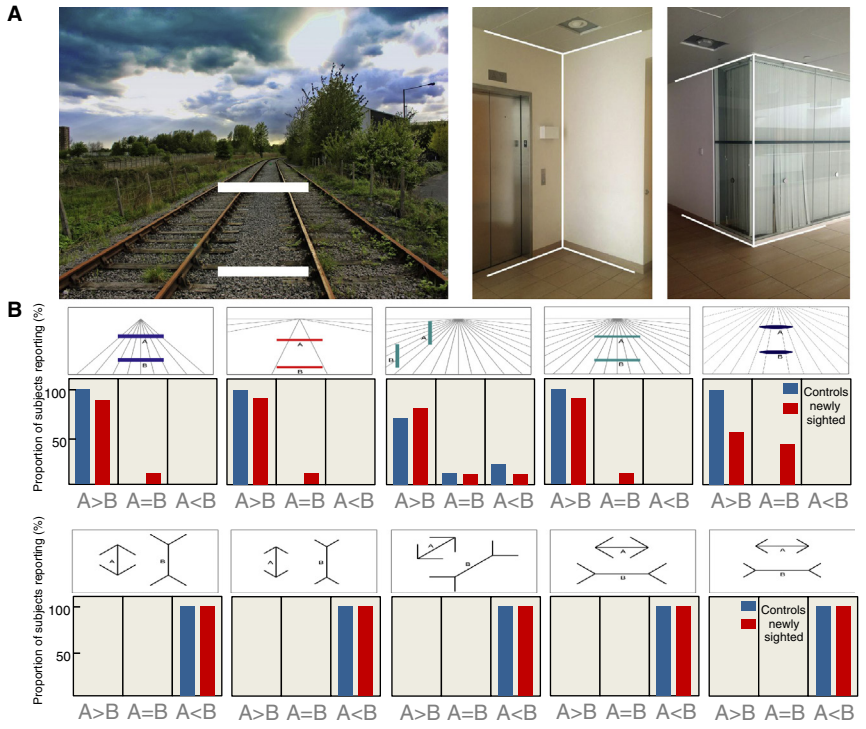

Figure 1. The susceptibility of newly-sighted individuals to visual illusions.
(A) The Ponzo and Müller-Lyer illusions superimposed on real images to indicate how learned perspective cues, as proxies for distance, may be the source of the effects. (Images after [5]; the railroad tracks image is by Darren Lewis and is in the public domain). (B) Results from normally sighted and newly sighted subjects on multiple displays. In each of these displays, the two lines being compared (denoted 'A' and 'B') are actually of identical length. Data are represented as the proportion of subjects (%) reporting each type of response.

