COMMENTARY

Making big cells: One size does not fit all

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During cell division, the genome is fully duplicated and then segregated to two daughter cells. In some tissues, however, cells repeatedly duplicate their genome and grow without dividing. This process results in large cells with many copies of their genome, a state known as polyploidy. In polyploid cells of the fruit fly *Drosophila*, DNA replication is not complete, which results in a relatively lower DNA copy number of specific genomic loci. In PNAS, Sher et al. show that in two different polyploid cell types of mice the entire genome is fully duplicated, unlike in *Drosophila*, and that this organismal difference may be explained by different levels of expression of the genes required for DNA replication (1). This report from Sher et al. reveals how these variant polyploid cell cycles can differ among organisms and tissues, but also uncovers many key similarities between flies and mammals that provide clues to the regulation of these enigmatic cell cycles.

Developmental Variations on the Cell Cycle Theme: Endocycles and Endomitosis

A common type of polyploid cycle is called the endocycle, which is comprised of alternating DNA synthesis (S) phases and gap (G) phases without chromosome segregation during a mitotic (M) phase or cell division (cytokinesis) (Fig. 1) (2). The endocycle is a normal developmental cell cycle variation that occurs widely, including in single-celled protists, plants, and humans. A related polyploid cell cycle is known as endomitosis, during which cells enter but do not complete mitosis nor divide (Fig. 1). During most polyploid cell cycles, the genome is duplicated only once per S phase. Rare exceptions to this rule occur in some endocycling cells, where a few gene loci repeatedly rereplicate to support a specific cell function, a process known as developmental gene amplification (3). Much remains unknown about the diversity of cell cycle mechanisms that lead to polyploidy and, in many cases, how an increase in DNA content and cell size supports cell function. Nonetheless, there have been some major advances recently, and I refer the interested reader to two excellent reviews (2, 4).

Polyploid Cells Differ in their Completion of Endoreplication

Polyploid cycles have been intensively studied in *Drosophila*, where cells of many tissues endocycle. Many heterochromatic loci, however, are not duplicated during every endocycle S phase, which results in their progressive underrepresentation relative to fully replicated euchromatic loci (5, 6). Although most of these underreplicated heterochromatic DNA are large blocks of pericentric short repeats, some gene loci with silencing chromatin marks are also underreplicated (7, 8). Evidence indicates that DNA replication is incomplete in all *Drosophila* polyploid cells examined, but that its extent can differ among loci and tissues (8–10).

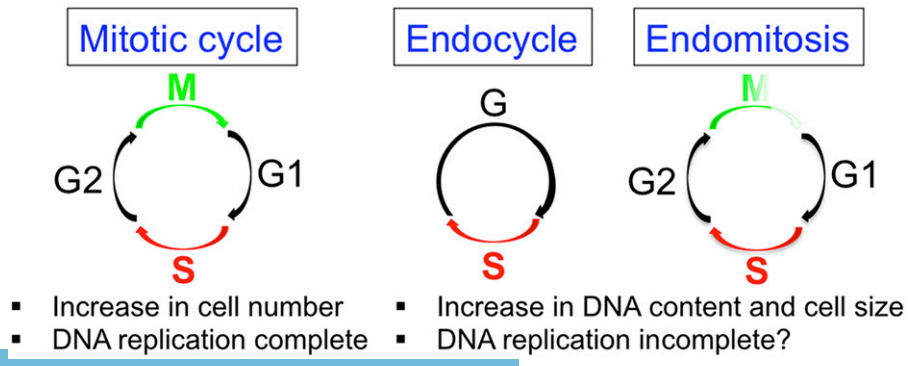

Fig. 1. The canonical mitotic cell cycle, endocycle, and endomitosis.

In PNAS, Sher et al. use array comparative genomic hybridization to examine two polyploid cell types in mice: endocycling trophoblast giant cells (TGCs) of the placenta, and endomitotic megakaryocytes (MKs), the precursors to blood platelets (1). Their analysis does not reveal developmental amplification of genes that are highly expressed and important for TGC and MK function. Surprisingly, unlike *Drosophila*, there was also no evidence for incomplete DNA replication. Because the array only contained probes for euchromatic loci, the authors evaluated DNA copy number at three heterochromatic loci by quantitative real-time PCR, which revealed that they are also fully replicated. Although it remains possible that other heterochromatic loci are not fully replicated, these results indicate that polyploidization cycles in TGCs and MKs differ from those in *Drosophila*, where large segments of the genome are severely underrepresented.

Completing Endoreplication: DNA Replication Genes, Cell Cycle Regulation, and Epigenomic Status

To explore what causes the difference between fly and mouse endoreplication, Sher et al. (1) analyze the expression of protein coding and microRNA genes in TGCs and MKs by microarray and RNA-Seq. A previous study in *Drosophila* found that many DNA replication genes are expressed at lower levels in endocycling cells than mitotic cycling cells, including genes that encode proteins required at replication origins and forks (11). Strikingly, both TGCs and MKs did not have the same drastic reduction in expression of DNA replication genes, perhaps explaining why DNA replication is more complete in mouse than fly. As Sher et al. (1) discuss, endocycle S phase may be slower in some *Drosophila* cells than mouse TGCs, and previous comparisons of cells from different *Drosophila* tissues suggested that the replication fork rate is 10 times slower and S phase up to several-fold longer in endocycling than mitotic cycling cells (12–14). Recent results indicate that underreplicated regions have an even slower replication fork

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rate and a paucity of active replication origins (15).

These data suggest, therefore, that the proteins that are required at replication origins and forks may be limiting in *Drosophila* for endoreplication of heterochromatin. Previous data, however, suggested that it is the altered oscillations of Cyclin E/cyclin-dependent kinase 2 (CDK2) activity during endocycles that causes underreplication (9). Heterochromatic loci normally replicate during late S phase of a mitotic division cycle, but during endocycles, Cyclin E/CDK2 activity drops and endocycle S phase ends before these heterochromatic loci are duplicated (9). Therefore, a cogent model is that although the *Drosophila* endocycle S phase is relatively long in duration, DNA replication is slower, and a drop in Cyclin E/CDK2 activity truncates the S phase before the duplication of late-replicating heterochromatin. Thus, underreplication may be determined by the interplay among replication protein levels, oscillations of CDK2 activity, and the epigenomic status of a locus in a specific cell type. An important test will be to identify which replication proteins are truly limiting for late replication in *Drosophila*, and determine whether fork rate and origin density differ between fly and mouse polyploid cells.

Mouse and *Drosophila* Polyploid Cycles: Different but Similar

Although Sher et al. (1) focus on the differences between *Drosophila* and mouse polyploid cycles, their data also uncover many similarities. One of the most notable similarities is the transcriptional repression of multiple genes required for mitosis and cytokinesis (1, 11, 16, 17). Two recent reports indicated that mouse TGCs and polyploid liver cells both have lower expression of genes required for mitosis and cytokinesis, many of which are also repressed during *Drosophila* endocycles (11, 18, 19). Sher et al. find that some of the mitotic genes are not as repressed in the endomitotic MKs, suggesting that this may distinguish endocycles from endomitosis (1).

The lower expression of mitotic genes is part of a larger similarity between mouse and *Drosophila* polyploid cycles: the dampened expression of genes regulated by the E2F family of transcription factors. In *Drosophila*, this dampened E2F transcriptome includes the DNA replication genes as well as the mitotic and cytokinesis genes (11, 18–21). Unlike TGCs, mouse liver cells also have lower levels of E2F-regulated DNA replication genes that are orthologous to those repressed during *Drosophila* endocycles (11, 19). This finding raises the question whether the genome is fully duplicated during liver endoreplication. Transcriptome analysis has revealed other similarities between mouse and *Drosophila* polyploid cycles, including altered expression of genes for metabolism and apoptosis (1, 11, 18, 19). Indeed, mouse TGCs and *Drosophila* endocycling cells both repress the apoptotic response to DNA damage (22–24). Thus, there are fundamental similarities and differences among polyploid cycles in different cell types of mouse, fly, and other organisms.

Cell Polyploidization: Variations on a Cell Cycle Variation

An open question is whether the difference between insects and mammals that Sher et al. (1) describe will apply to other tissues and organisms. It is possible that other polyploid cell types in mammals underreplicate to some degree, whereas some recently described polyploid cell types of *Drosophila* may have more complete replication (25, 26). Indeed, genome duplication is complete during the first five endocycles of *Drosophila* ovarian nurse cells, and some heterochromatic DNA may be fully replicated in salivary glands of other insects (10, 27). Similar to mouse, endocycling leaf cells of *Arabidopsis thaliana* also fully duplicate their heterochromatic DNA (28). Thus, there is a diversity of endoreplication programs among tissues as well as organisms. The report from Sher et al. (1) is an important advance for understanding this diversity. It remains unclear, however, whether underreplication fulfills a specific purpose or is simply a result of altered regulation of the polyploid cell cycle. Nonetheless, given that some cancer cells inappropriately engage an endocycle program, further investigation into underreplication during development may reveal new mechanisms that contribute to genome instability in the cancer cell (29). The genomic data from Sher et al. (1) is a rich treasure trove for future research into these polyploid cell cycle variations, and demonstrates that when it comes to making big cells, one size certainly does not fit all.

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