The expanding implications of polyplody

Kevin P. Schoenfelder and Donald T. Fox

Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27710

Polyplody cells, which contain more than two genome copies, occur throughout nature. Beyond well-established roles in increasing cell size/metabolic output, polyplody can also promote nonuniform genome, transcriptome, and metabolome alterations. Polyplody also frequently confers resistance to environmental stresses not tolerated by diploid cells. Recent progress has begun to unravel how this fascinating phenomenon contributes to normal physiology and disease.

Polyplody cells are found in diverse taxa (Fox and Duronio, 2013; Edgar et al., 2014), and in fact entire organisms can be polyplod, or polyplody cells can exist in otherwise diploid organisms (endopolyploidy). In humans, polyplody cells are found in critical tissues, such as liver and placenta. A general term often used to describe the generation of polyplody cells is endoreplication, which refers to multiple genome duplications without intervening division/cytokinesis. We refer the reader to several recent reviews describing polyplody as a fascinating phenomenon contributes to normal physiology and disease.

One long-appreciated polyplody genome modification is underreplication of specific genome regions (Fig. 1 A; Gall et al., 1971; Endow and Gall, 1975). Underreplication often occurs in polyplody cells with giant polyplody chromosomes, such as in dipteran salivary glands (Belyaeva et al., 1998; Fox and Duronio, 2013; Edgar et al., 2014) or mammalian placental giant trophoblast giant trophoblast (Hannibal et al., 2014). Interestingly, underreplicated regions include gene-containing regions (Belyakin et al., 2005; Nordman et al., 2011; Sher et al., 2012; Yarosh and Spradling, 2014).

Recent work in Drosophila melanogaster has illuminated that each endoreplication creates unique genomic deletions and rearrangements (Yarosh and Spradling, 2014), generating sequence heterogeneity, both at the same locus within the same cell, and among different cells in the same tissue. Underreplicated regions can exhibit decreased gene expression (Nordman et al., 2011; Hannibal et al., 2014), but an exception to this trend occurs in Drosophila fat body (Nordman et al., 2011). It remains to be seen whether transcript-enhancing or transcript-creating gene rearrangements occur in these underreplicated regions, as in ciliates (see next paragraph). In diploid cells, similar incomplete DNA replication can occur at “fragile” chromosome sites, which leads to chromosome breakage. Such breaks are viewed as detrimental to the diploid cell (Laird, 1989; Mazouzi et al., 2014). Thus, polyplody underreplication may represent a conserved mechanism of extensive somatic genome alteration used preferentially by polyplody cells.

Even more extreme are the deletions and rearrangements during polyplody in ciliate protozoans such as Oxytricha trifallax. This organism (and other ciliates) contains a haploid germline micronucleus (MIC) and a polyplody macronucleus (MAC). The MAC is formed by extensive fragmentation/rearrangement of the MIC genome into ~16,000 gene-sized “nanochromosomes” (Lauth et al., 1976; Dawson et al., 1984; Chen et al., 2014). Through action of long noncoding RNAs (Nowacki et al., 2008), transposases (Nowacki et al., 2009), and Piwi-interacting RNAs (Fang et al., 2012), O. trifallax produc- tively unscrambles thousands of inactive MIC genes into functional MAC genes (Fig. 1 B). This process removes over 90% of the MAC genome, including germline transposons (Swart et al., 2013). A single MAC-destined sequence stretch in the MIC can be alternatively processed into up to five different MACs.
nanochromosomes (Chen et al., 2014), and local underreplication occurs on chromosomes in *O. trifallax* and other hypotrich ciliates (Baird and Klobutcher, 1991; La Terza et al., 1995; Dönhoff and Klein, 1996; Frels et al., 1996). Thus, genome unscrambling creates genetic heterogeneity in these polyploid cells. Although the purposes of underreplication and associated deletions in polytene cells have yet to be fully appreciated, their recurring nature and association with gene alterations suggest that they could play an important role in altering cellular function.

Yet another recurring form of polyploid genome alteration occurs in endopolyploid cells capable of mitosis. The ability of some endopolyploid cells to divide shows that polyploidization cannot be universally characterized as a means of tissue growth for nonproliferative tissues. During these polyploid divisions, instead of evenly partitioning the genome, daughter cells are frequently created with chromosome number imbalances, or aneuploidy. Although the association between polyploidy and aneuploidy was originally appreciated in cases of aberrant polyploidy (Fujiwara et al., 2005; Shi and King, 2005; Storchová et al., 2006; Davoli and de Lange, 2012), it’s now known that naturally occurring mouse liver hepatocytes and *Drosophila* and *Culex pipiens* (mosquito) rectal papillar cells generate aneuploidy during polyploid divisions (Duncan et al., 2010; Fox et al., 2010; Schoenfelder et al., 2014).

This aneuploidy can occur in part by formation of multipolar mitotic spindles (Fig. 1 C), which arise from centrosome amplification during endoreplication. In *Drosophila*, such aneuploid divisions are perfectly capable of supporting development/function of polyploid rectal papillae (Schoenfelder et al., 2014). Recent single-cell sequencing suggests the unstressed liver may have only a low level of aneuploidy (Knouse et al., 2014), but other work (see section But size DOES matter) suggests hepatocyte aneuploidy may be selectively amplified in liver disease (Duncan et al., 2012). Given the many negative outcomes attributed to aneuploidy (e.g., cancer and birth defects), it’s interesting that aneuploidy may be tolerated or even selected for in mitotic polyploid tissues.

**Polyplody variably alters the transcriptome**

In addition to genome alterations, polyploidy can substantially alter the transcriptome. One simple model for how polyploidy may alter cellular function is by enabling increased transcription on a per cell basis, with mRNA increases proportional to overall genome content. Instead, polyploidization is commonly accompanied by uneven, locus-specific transcriptional alterations, driven at least in part by epigenetic changes.

Through comparison of diploid and polyploid plants of the same species (Coate and Doyle, 2010), or across the genome of a *Drosophila* cell line with heterogeneous ploidy (Zhang et al., 2010), it was found that a simple linear relationship does not universally exist between chromosome ploidy and transcript levels. As discussed next, many polyploid transcriptional changes are a result of increased cell size, and not caused by ploidy per se, and thus are not proportional to gene copy number (Wu et al., 2010; Miettinen et al., 2014).

Keeping in mind that polyploid cells often bend the rules, it’s important to point out that polyploid genome alteration can lead to locus-specific gene copy number increases, which often facilitate transcriptional up-regulation of those genes. Locus-specific amplification of gene expression (Fig. 1 A) is accomplished in the developing salivary gland of the fly *Scara coprophila* (Wu et al., 1993) and the ovarian follicle of *Drosophila* (Spradling and Mahowald, 1980; Calvi et al., 1998; Claycomb and Orr-Weaver, 2005). In *Drosophila*, this amplification is accomplished by local overreplication (gene amplification) of the
eggshell gene regions, which in many cases is developmentally timed with the need for rapid egg shell synthesis. However, not all follicle cell gene amplification correlates with an obvious developmental need for more transcription (Kim and Orr-Weaver, 2011). We speculate that, just as heterogeneity associated with gene amplification can promote selective advantages in cancer (Jacot et al., 2013) or in poxviruses during infection (Elde et al., 2012), amplified loci in polyploid cells may introduce additional, potentially beneficial genetic heterogeneity during successive genome duplications.

One mechanism by which polyploidy can confer specific gene expression changes is at the epigenetic level. Many of these changes may arise during the switch from canonical cell cycles to endoreplication cycles. For example, it was recently shown that *Drosophila* follicle cells decrease levels of the histone lyase demethylase Lsd1 at a developmentally programmed mitosis-to-endoreplication switch. This decrease in Lsd1 is accompanied by a rise in histone H3 lysine 4 (H3K4) methylation levels and a marked decrease in epigenetic plasticity during the transition to polyploidy (Lee and Spradling, 2014).

In mammals, endoreplication also increases H3K4 methylation by countering another epigenetic repression mechanism: X chromosome inactivation (XCI). In the giant polyploid trophoblast cells of the placenta, many X-linked genes escape XCI and exhibit biallelic expression. Interestingly, the lack of XCI is not caused by a failure to recruit Xist mRNA, a well-known XCI regulator. Instead, the escape from XCI may be a result of an unusual chromatin status in polyploid giant trophoblasts, which contains an abundance of not only active H3K4 marks but also the repressive H3K27me3 mark (Corbel et al., 2013). Fascinatingly, this mixture of active and repressive marks mirrors the “bivalent chromatin” signature of embryonic stem cells, which is thought to facilitate gene repression while maintaining key genes in a poised state (Bernstein et al., 2006). Perhaps polyploid trophoblasts use bivalent chromatin to bypass XCI, thus achieving repression while retaining the ability to enhance specific X chromosome transcripts in response to external signals such as hormones in the placental environment. Collectively, the aforementioned examples highlight that the relationship between total cellular ploidy and RNA levels can be far from linear, underscoring that it’s too simplistic to think of a polyploid cell as a big diploid cell.

**When times get rough, it’s good to be polyploid**

The aforementioned examples highlight the incredible biological diversity and molecular repertoire of polyploid cells. From such diversity, are there common threads in terms of polyploidy’s function? Accumulating evidence suggests there may be, in response to cellular stresses.

All organisms face cellular stress from DNA damage. A recurring theme is that polyploidy promotes DNA damage insensitivity. A priori, this may make sense because extra genome copies mean extra repair templates and higher damage doses needed to cause detrimental genome changes. For example, in *Arabidopsis thaliana*, UV exposure drives increased endopolyploidy in leaves (Gegas et al., 2014). But beyond extra gene copies, specific DNA damage response alterations occur in many polyploid cells. The polyploid bacterium *Deinococcus radiodurans* is highly resistant to DNA damage and efficiently reassembles its genome even if it has been shattered by excessive double-strand DNA breaks. This involves extended synthesis-dependent strand annealing, a recently identified DNA repair mechanism (Zahradka et al., 2006).

Multiple endoreplicated polyploid *Drosophila* tissues exhibit DNA damage insensitivity (Mehrotra et al., 2008), caused in part by epigenetic silencing of proapoptotic genes and enhanced proteolysis of the DNA damage regulator p53 (Zhang et al., 2014). p53 is also down-regulated in mammalian placental trophoblasts (Soloveva and Linzer, 2004), and p53 inactivity increases proliferation of mammalian polyploid cells in many contexts (Wong and Stearns, 2005; Ganem et al., 2014). The kinase Chk1 is also inactivated in mammalian trophoblasts, rendering these cells insensitive to DNA damage (Ullah et al., 2008). Thus, diverse mechanisms tie polyploidy to inhibition of DNA damage signaling.

Outside of DNA damage, pathogenic fungi also use polyploidy to adapt to host stresses. The fungal pathogen *Cryptococcus neoformans* increases cell size/genome content during human lung infection. These “Titan cells” resist phagocytosis by immune host cells (Zaragoza et al., 2010; Okagaki and Nielsen, 2012). *Candida albicans*, a prevalent human pathogenic fungus, polyploidizes in response to the antifungal drug fluconazole (FLC; Harrison et al., 2014). FLC exposure leads first to tetraploidy followed by missegregated chromosomes, creating FLC-resistant aneuploid progeny. In contrast to the oft-described negative attributes of aneuploidy, many FLC-resistant aneuploids show little fitness cost.

In nonpathogenic *Saccharomyces cerevisiae*, polyploid-induced genome change can also lead to selection of beneficial genotypes under stress conditions. Aneuploidy in yeast derived from a triploid parent drives proteomic changes not just of genes located on the aberrant chromosomes (Pavelka et al., 2010). These aneuploidies sometimes result in fitness benefits in response to a battery of stressful growth conditions. Both aneuploidy and an increased mutation rate can benefit polyploid cells under evolutionary pressure. A recent in vitro evolution study found that in response to growth on a poor carbon source, tetraploid *S. cerevisiae* undergo more rapid adaptation than diploids, as a result of more frequent beneficial mutations and stronger fitness effects (Selmecki et al., 2015). Although the aforementioned studies focused on ploidy variation in a single nucleus, recent study of the multinucleate fungus *Ashbya gossypii* subjected to a panel of stress stimuli found that polyploid cells can revert to a haploid state (Anderson et al., 2015). Collectively, it’s clear polyploidy is a nonneutral player under conditions of selective pressure.

In whole tissues, injury/disease may also drive polyploid-induced tissue adaptation. The liver is well known to regenerate in response to injury. Over half of adult mouse hepatocytes are estimated to be polyploid (4–16N), and these polyploid cells can undergo aneuploid-prone mitosis (Duncan et al., 2010). In a genetic liver damage model, chromosome 16 aneuploidy (loss) was highly enriched in expanding, disease-resistant regions of...
the liver. Chromosome 16 encodes the homogentisic acid dioxygenase gene, loss of which confers resistance in this particular liver damage model (Duncan et al., 2012). These results suggest aneuploidy through polyploid hepatocyte mitosis enables the liver to adapt to injury/infection.

In the absence of cell division, polyploidization is also implicated in active wound-healing responses in both invertebrates and mammals. A puncture injury to the Drosophila abdomen causes epithelial cells near the wound to fuse into a polyploid syncytium to reestablish the epithelium and also triggers endoreplication to increase the ploidy of the repairing tissue (Losick et al., 2013). Endoreplication also occurs in the injured/repairing Drosophila hindgut pylorus and ovarian follicle epithelium (Losick et al., 2013; Tamori and Deng, 2013). The findings in these fly tissues bear striking similarity to the ability of the mammalian liver to fully regenerate under conditions where cell division is impaired (Davoli and de Lange, 2012; Diril et al., 2012). It will be interesting to determine what physiological conditions activate polyploidization instead of cell division for purposes of tissue repair. The aforementioned studies highlight an impressive array of stresses in which polyploidy plays an important role.

But size DOES matter!
The previous sections of this article highlight ways in which polyploid cells go beyond being just bigger. However, the big size of polyploid cells can’t be ignored, as polyploidy is a well-recognized phenomenon. In Xenopus embryos, it was found that altering megakaryocyte ploidy deregulates platelet production (Murone et al., 1998; Chagraoui et al., 2011).

In addition to metabolic and mechanical alterations, a third way in which size may contribute to the altered biology of polyploid cells pertains to the size of intracellular structures. One example of how size impacts biology of intracellular structures pertains to the mitotic spindle. By both experimentally altering cytoplasmic volume in a Xenopus laevis extract system and by observing cells in intact Xenopus embryos, it was found that although spindle length scales perfectly with cytoplasmic volume in smaller cells, this does not hold true in large cells/cytoplasmic volumes (Good et al., 2013). This uncoupling of spindle size scaling in large cells may explain why mitotic spindles in polyploid yeast are strikingly different in structure from those of diploid yeast. This spindle structure alteration is proposed to contribute to the increase in erroneous spindle–chromosome interactions that lead to chromosome missegregation in polyploid yeast cells (Storchová et al., 2006). Studies such as these emphasize that increased size—a common property of polyploidy—can alter cellular function in biologically important ways.

Conclusion
When it comes to polyploidy, the aforementioned studies highlight that size matters, but it is clearly not all that matters. Polyploidy can be accompanied by extensive alterations to the genome, epigenome, transcriptome, and metabolome, in a manner that does not simply resemble a larger version of their diploid relatives. Mechanisms generating these alterations, and their outcomes, are as wide ranging as the organisms/tissues in which they occur. Yet, common themes are emerging. Similar metabolic changes may occur in many polyploid cells, driven not...
directly by ploidy increase but instead by increased cell size. An enhanced tolerance to stresses such as DNA damage has emerged as a recurring advantage of diverse polyploid cells. Relative to their diploid counterparts, polyploid cells engage in frequent, well-tolerated genome alterations (Fig. 1), and similar polyploid genome alterations are found in diverse organisms.

Determining the function/implication of polyploid genome/transcriptome alterations remains a key challenge in the field. Given the expanding picture of polyploid biology, specific transcriptional/metabolic changes or increased genomic heterogeneity (Fig. 1 D) after polyploidization may facilitate stress tolerance in many endopolyploid tissues and in cases of whole organismal polyploidy (Fig. 2). It will be particularly interesting to see whether polyploid genome alteration is a general mechanism for selection of stress resistant phenotypes.

Continued study of polyploid cells may also impact human disease. Aneuploidy is known to derive from division of polyploid cancer cells (Ganem et al., 2007; Davoli and de Lange, 2011). Furthermore, the conserved changes described in some large cells bear similarity to the Warburg-like metabolism described in many cancer cells (Miettinen et al., 2014). Given the recent confirmation that polyploidy is a recurring feature in diverse human cancers (Zack et al., 2013), understanding properties that differentiate diploid and polyploid states will be beneficial for developing new therapies. If recent progress in this exciting area of cell biology is any indication, it’s clear that unraveling new functions of polyploidy could make a sizeable impact.

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