Accepted Manuscript online: 2023-11-15 Article published online: 2023-12-15

The Ploidy State as a Determinant of Hepatocyte Proliferation

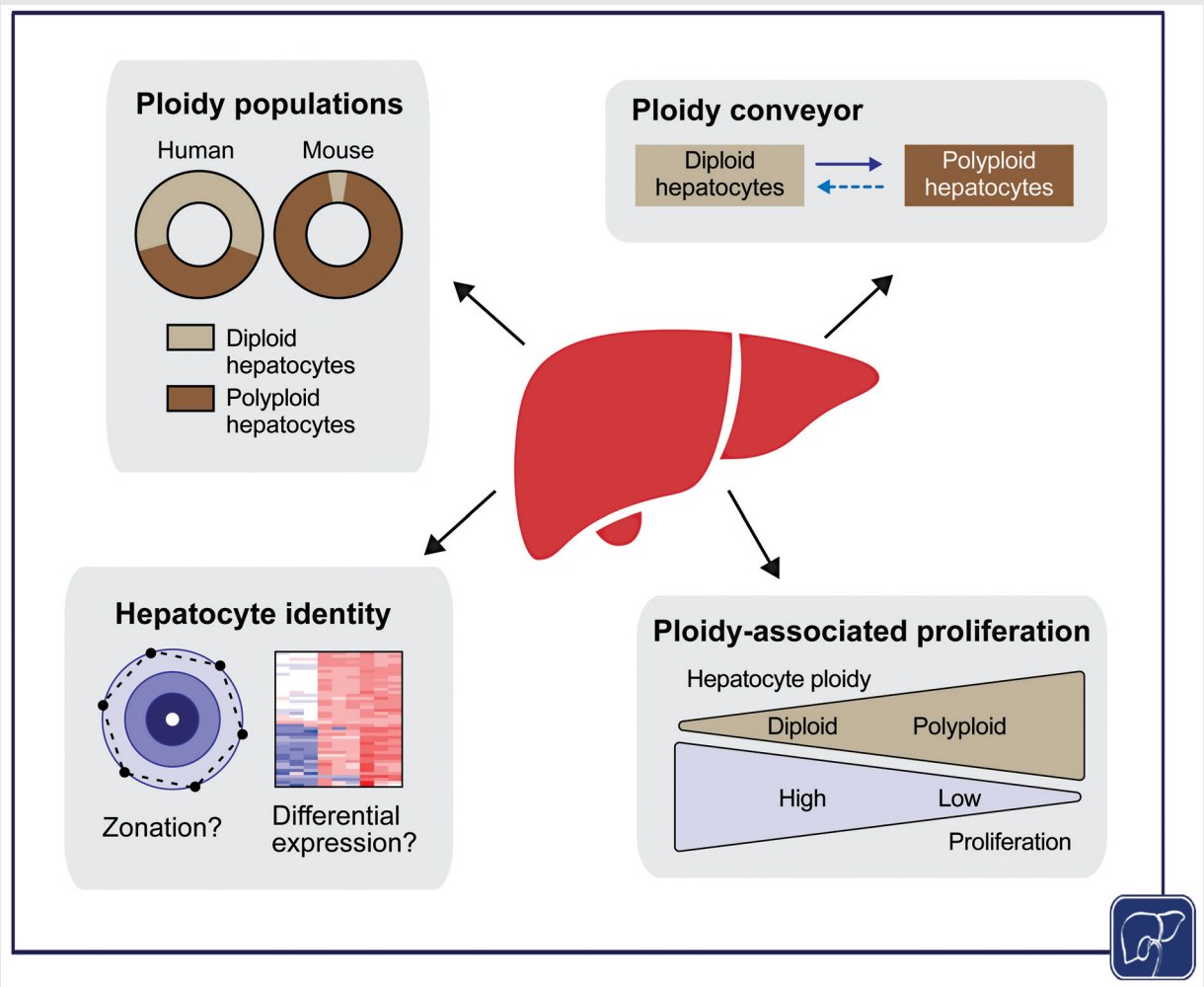
Sierra R. Wilson, BA[1] Andrew W. Duncan, PhD[1]

[1] Department of Pathology, McGowan Institute for Regenerative Medicine, Pittsburgh Liver Research Center, University of Pittsburgh, Pittsburgh, Pennsylvania

Semin Liver Dis 2023;43:460–471.

Address for correspondence Andrew W. Duncan, PhD, Department of Pathology, McGowan Institute for Regenerative Medicine, Pittsburgh Liver Research Center, University of Pittsburgh, 450 Technology Drive, Suite 300, Pittsburgh, PA 15219 (e-mail: duncana@pitt.edu).

Graphical Abstract



accepted manuscript online
November 15, 2023

Thieme Medical Publishers, Inc.,
333 Seventh Avenue, 18th Floor,
New York, NY 10001, USA

DOI https://doi.org/10.1055/a-2211-2144.
ISSN 0272-8087.

Abstract

The liver's unique chromosomal variations, including polyploidy and aneuploidy, influence hepatocyte identity and function. Among the most well-studied mammalian polyploid cells, hepatocytes exhibit a dynamic interplay between diploid and polyploid states. The ploidy state is dynamic as hepatocytes move through the "ploidy conveyor," undergoing ploidy reversal and re-polyploidization during proliferation. Both diploid and polyploid hepatocytes actively contribute to proliferation, with diploids demonstrating an enhanced proliferative capacity. This enhanced potential positions diploid hepatocytes as primary drivers of liver proliferation in multiple contexts, including homeostasis, regeneration and repopulation, compensatory proliferation following injury, and oncogenic proliferation. This review discusses the influence of ploidy variations on cellular activity. It presents a model for ploidy-associated hepatocyte proliferation, offering a deeper understanding of liver health and disease with the potential to uncover novel treatment approaches.

Keywords

- polyploidy
- liver regeneration
- liver injury
- liver repopulation
- ploidy conveyor

Lay Summary

Hepatocytes, the primary functional cells of the liver, are characterized by variations in nuclear content. Most hepatocytes have single pairs of each chromosome (diploid hepatocytes), but many have extra chromosome sets (polyploid hepatocytes). The function of diploid and polyploid hepatocytes is poorly understood. However, recent work demonstrated that both diploid and polyploid hepatocytes contribute to liver growth and regeneration, but diploids are capable of accelerated growth. Thus, diploid hepatocytes play a dominant role in liver maintenance (as they replace aging cells), replacement of damaged hepatocytes after injury, and even in the development of liver cancer. Deciphering how diploid and polyploid hepatocytes are regulated and function is essential for developing new therapies for liver disease treatment.

Chromosome Variations in the Liver

Cellular ploidy refers to the number of complete chromosome sets in a cell. Most mammalian cells are diploid and contain homologous pairs of each chromosome, but there are also cells with higher chromosomal content. Polyploid cells have increased sets of chromosomes beyond the diploid state. In humans, diploid cells (2n) have 23 chromosome pairs for a total of 46 chromosomes, while polyploid cells can have 92 (4n, tetraploid), 184 (8n, octaploid), or even greater numbers of chromosomes. Polyploidy is frequently associated with genomic instability and cancer but is also found in healthy mammalian tissues, including cardiac myocytes, megakaryocytes, giant trophoblasts, skeletal muscles, and hepatocytes.[1–3] Polyploidy is distinct from numerical aneuploidy, where a cell loses or gains individual chromosomes ($2n \pm 1$). Whole-organism aneuploidy is typically detrimental, impairing development and function, but somatic aneuploidy is more complex. While aneuploidy is considered a hallmark of cancer, there are exceptions.[4–6] For example, aneuploidy is associated with tumor suppression in fibroblasts from individuals with Down's Syndrome through reduced proliferation.[6,7] Thus, the consequences of polyploidy and aneuploidy depend on the mechanism of formation and tissue type.

Hepatocytes are characterized by remarkable chromosomal variations that include polyploidy and aneuploidy. Polyploidy was recognized in the liver over a century ago, and hepatocytes have since emerged as some of the best-characterized mammalian polyploid cells.[8] The liver is highly polyploid, with polyploid hepatocytes comprising more than 90% of the hepatocyte population in adult mice and 25 to 50% in humans.[9,10] Numerical aneuploidy is also observed in liver tissue, where 5 to 50% of hepatocytes are aneuploid, depending on how aneuploidy is measured, in healthy mouse and human liver tissues.[11,12] Notably, this hepatic aneuploidy randomly affects all chromosomes, generating a genetically diverse population.[12] Chromosomal variations have a wide-ranging impact on hepatocyte identity and function.[12,13] Several recent and extensive reviews describe the mechanisms of hepatic polyploidy and the functions of ploidy populations in homeostasis and disease.[14,15] This review will focus primarily on the distinct roles of diploid and polyploid hepatocytes during homeostasis and regeneration.

Mechanisms of Hepatocyte Control

The Ploidy Conveyor

Hepatic polyploidy is classified by the DNA content per nucleus (nuclear ploidy) and the number of nuclei per cell (cellular ploidy).[15] For example, a tetraploid cell could have a single 4n nucleus or two 2n nuclei (►**Fig. 1**). Polyploidization occurs by several cellular mechanisms, including endoreplication, mitotic slippage, and cellular fusion, but physiological polyploidization of hepatocytes predominately occurs by acytokinetic mitosis.[15–17] Hepatocytes are exclusively diploid at birth and undergo gradual physiological polyploidization during postnatal

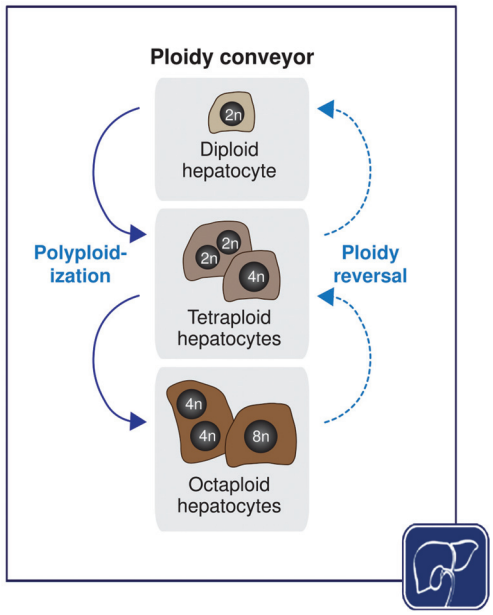


Fig. 1 The ploidy conveyor model of hepatocytes. Diploid hepatocytes are mononucleate with a single 2n nucleus. Polyploid hepatocytes can be mono- or binucleate, where tetraploids have a pair of 2n nuclei or a single 4n nucleus, octaploids have a pair of 4n nuclei or a single 8n nucleus, and so on. The ploidy conveyor model incorporates hepatic ploidy flexibility as hepatocytes proliferate. Acytokinetic cell division increases hepatic ploidy (physiological polyploidization), while multipolar cell division reduces hepatic ploidy (ploidy reversal).

development. First, a subset of proliferating diploid hepatocytes fail to complete cytokinesis, producing binucleate tetraploid daughter cells.[18,19] Second, both the mono- and binucleate subsets of hepatocytes will continue to undergo DNA replication and mitosis, either with complete cytokinesis (generating mononucleate diploid or polyploid cells) or with acytokinetic mitosis (generating binucleate polyploid cells). This way, diploid, tetraploid, octaploid, and even higher ploidy state hepatocytes are produced, with polyploid hepatocytes existing in mononucleate and binucleate forms. Hepatic polyploidy is also reversible, where polyploid hepatocytes produce daughter cells with one-half ploidy that can subsequently re-polyploidize.[9,20] The process of hepatic polyploidization, ploidy reversal, and re-polyploidization is described as the "ploidy conveyor" (►**Fig. 1**).[9,21]

Physiological Polyploidization and Ploidy Reversal

Hepatic polyploidization begins during postnatal development, coinciding with the weaning phase in rodents.[22] This period is characterized by significant shifts in feeding patterns, alterations in hormone levels, and changes in metabolic pathways that impact key polyploidization regulators. For example, insulin signaling changes dramatically during weaning and is a key factor in generating binucleate hepatocytes.[23] In rats, impaired insulin signaling reduced the formation of binucleate tetraploid hepatocytes, while increasing insulin increased the formation of tetraploid hepatocytes.[22,23] The cellular effects of insulin are mediated by the PI3K/AKT pathway, which antagonize RHOA and negatively regulate actin cytoskeleton polarization (►**Fig. 2A**). Also, highly expressed during postnatal development, E2F7 and E2F8 are transcription factors influencing polyploidization through cell cycle regulation. Specifically, E2F7 and E2F8 antagonize E2F1 and negatively regulate cytokinesis genes; thus, loss of E2F7 and E2F8 promotes successful cytokinesis of proliferating diploid hepatocytes.[24,25] Mice with a liver-specific double knockout of *E2f7* and *E2f8* have livers that exhibit normal function through 6 to 9 months but are significantly depleted of binucleate and polyploid hepatocytes.[24–27] MicroRNAs (miRNAs) are another regulator of hepatocyte polyploidization. miR-122 is the most abundant miRNA in adult livers, with expression in mice spiking during postnatal development. miR-122 affects polyploidization by negatively regulating procytokinesis genes (*RhoA*, *Mapre1*, and *Iqgap1*), which impairs the formation of the centralspindlin complex and inhibits cytokinesis.[28,29] *Mir122* knockout mice are significantly depleted of polyploid hepatocytes.[29] Hepatocyte polyploidization is also regulated by the PIDDosome complex. The PIDDosome is a multiprotein complex activated by extra centrosomes in polyploid cells that induces p21 and restricts proliferation and hyperpolyploidization.[14,30–32] The absence of the PIDDosome leads to unrestrained polyploidization and hyperpolyploidy, interestingly contributing to tumor resistance.[32] Finally, several other genes have been associated with altered cell cycle regulation and changes in hepatocyte ploidy (*FoxO3*, *Cdk1*, etc.), but their roles in liver polyploidization are poorly defined.[33,34]

Multiple overlapping pathways define the networks regulating polyploidy. For instance, the actin cytoskeleton (specifically RHOA) is destabilized by insulin and miR-122 activity.[29,35] The centralspindlin complex, necessary for cytokinesis, is inhibited by miR-122 and E2F7/E2F8 signaling.[24,29] Similarly, the expression of PIDDosome complex members, *Pidd1* and *Casp2*, is negatively regulated by E2F7 and E2F8.[36]

In contrast to physiological polyploidy, the signals regulating ploidy reversal are poorly defined.[9,17,20] Polyploid hepatocytes have extra centrosomes and form multipolar mitotic spindles early in mitosis. Centrosomes typically cluster to produce bipolar mitosis with two-way nuclear segregation. However, in some cases, the multipolar spindle persists, leading to multipolar cell division and the generation of daughter cells with one-half DNA content. For example, multipolar cell division by a tetraploid hepatocyte can form more than two nuclei, including those with diploid or near-diploid content, and successful cytokinesis generates diploid daughter hepatocytes (►**Fig. 2B**).

In summary, hepatocyte ploidy is incredibly dynamic. Physiological polyploidization primarily occurs through acytokinetic mitosis regulated by a complex network, including E2F family members, miR-122, and PIDDosome signaling. Ploidy reversal occurs by multipolar cell division of a polyploid hepatocyte, leading to the birth of diploid hepatocytes. The integration of physiological polyploidization and ploidy reversal is discussed below.

Pathological Polyploidization

An excessive accumulation of hepatic polyploidy has been linked to metabolic and oxidative stress. Mouse models of nonalcoholic fatty liver disease (NAFLD) and patients with

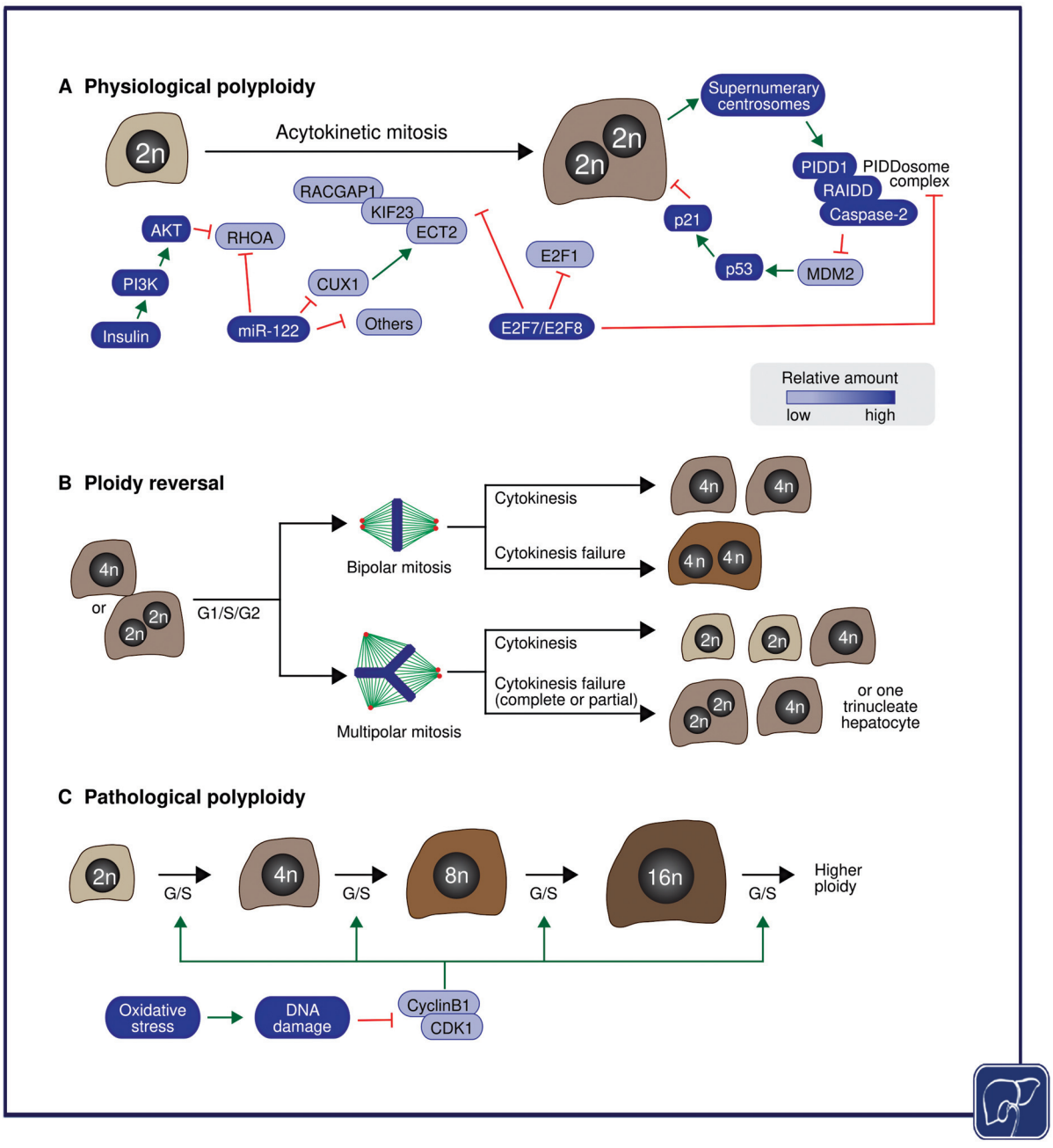


Fig. 2 Mechanisms of hepatic polyploidization and ploidy reversal. (**A**) Physiological polyploidization begins when a hepatocyte completes a cell cycle without cytokinesis, generating a binucleate cell. The formation of a binucleate tetraploid hepatocyte by a diploid hepatocyte is shown. This process is regulated by multiple networks, including insulin, miR-122, and E2F7/E2F8 that disrupt contractile ring formation, furrow ingression, and abscission. For example, RHOA, which promotes cleavage furrow ingression, is inhibited by insulin and miR-122. Similarly, miR-122 and E2F7/E2F8 inhibit the centralspindlin complex (comprised of ECT2, RACGAP1, and KIF23) that regulates cleavage furrow ingression and abscission. Once formed, polyploid hepatocytes have supernumerary centrosomes. Supernumerary centrosomes activate the PIDDosome, resulting in p21 expression and attenuating hyperpolyploidization and proliferation. (**B**) Ploidy reversal occurs when a polyploid hepatocyte undergoes multipolar mitosis to generate daughter cells with reduced nuclear content. In the example shown, a tetraploid hepatocyte (mononucleate or binucleate) progresses through the cell cycle with a bipolar mitosis to generate two tetraploid nuclei. Successful cytokinesis produces two mononucleate tetraploid hepatocytes, while cytokinesis failure generates a single binucleate octaploid. In contrast, multipolar mitosis (three-way nuclear segregation is shown, but four-way segregation is possible) generates three nuclei. Successful cytokinesis produces two diploid (or near-diploid) hepatocytes and a mononucleate tetraploid (or near-tetraploid) hepatocyte. Cytokinesis failure produces one or more polyploid hepatocytes. Signals that control hepatic multipolar cell division are poorly described. (**C**) Pathological polyploidy occurs by altered cell cycling when a hepatocyte alternates between the growth phase (G) and DNA synthesis (S) without mitosis. Signals regulating pathological polyploidy are poorly defined. However, oxidative stress-induced DNA damage can inhibit the CyclinB1/CDK1 complex and block mitosis entry, leading to successive rounds of G and S phases, forming mononucleate hepatocytes with highly polyploid nuclei.

nonalcoholic steatohepatitis exhibit increased levels of polyploid hepatocytes and significant enrichment in mononucleate cells with high nuclear content.[35] This distinctive ploidy distribution, termed *pathological polyploidy*, contrasts with physiological polyploidization that occurs during postnatal development. Oxidative damage is the primary driver of ploidy alterations in NAFLD. Hepatic oxidative damage results in a DNA damage response and inhibition of the CyclinB1/CDK1 complex, which is necessary for mitosis entry (►**Fig. 2C**). As a result, hepatocytes undergo atypical cell cycles where they replicate DNA, transiently arrest in G2, skip mitosis, and then re-enter the cell cycle. This cycle repeats, causing mononucleate polyploid hepatocytes to become even more polyploid. The function of these highly polyploid hepatocytes is uncertain, and further investigation is needed to establish whether they contribute to disease progression or resistance.[37]

Ploidy and Liver Function

Spatial Organization

In addition to their nuclear heterogeneity, hepatocytes have diverse functions. The liver is organized into repeating hexagonal liver lobules divided into three zones based on their orientation around the vessels.[38–41] Blood enters the tissue via the portal tract (periportal region = zone 1), flows through the liver sinusoids in the midzone region (zone 2), and drains into the central vein (pericentral region = zone 3), creating a gradient of oxygen, nutrients, and hormones that allows hepatocytes in different zones to have unique gene expression patterns and functions. For example, hepatocytes in the oxygen and nutrient-rich environment of zone 1 perform metabolic functions like beta-oxidation, gluconeogenesis, urea and protein synthesis, and lipid metabolism. Zone 2 serves as a primary source for new hepatocytes during homeostasis and regeneration.[42] Finally, hepatocytes in zone 3 perform glycolysis, xenobiotic biotransformation reactions, and glutamine synthesis.[42–44] Since the expression of many hepatocyte-specific genes is restricted to certain zones, the spatial distribution of diploid and polyploid hepatocytes may provide clues to their specialized functions.

Multiple studies have examined the distribution of ploidy populations within the liver lobule, but the conclusions vary widely (►**Table 1**). First, Tanami et al investigated the location of diploid and polyploid hepatocytes within the liver lobule. The ploidy state was determined by chromosome counting with DNA fluorescence in situ hybridization (FISH) along with membrane staining to distinguish mono- and binucleate cells. The location of each cell was determined based on the proximity to the central vein. Rapid polyploidization was observed 3 to 4 weeks after birth with enrichment of polyploids in the midlobule zone.[45] Second, Katsuda et al evaluated gene expression of zonal markers in rat hepatocytes separated by ploidy and performed bulk microarray analysis and single-cell quantitative reverse-transcription polymerase chain reaction (sc-qRT-PCR).[46,47] They found genes associated with zone 3 (*Glul*, *Cyp7a1*, *Slc1a2*) were enriched in diploid hepatocytes. In contrast, zone 1 (*Alb*, *G6pc*, *Tat*) genes were more highly expressed by polyploids, suggesting that diploid and polyploid hepatocytes localize to pericentral and periportal areas, respectively. Third, ploidy and zonation were investigated at the nuclear

Table 1 Conflicting reports of the zonal distribution of diploid and polyploid hepatocytes

Report	Species	Age	Tissue Processing	Ploidy determination	Zonation determination	Finding
Tanami et al[45]	Mouse	2 mo	Liver sections (frozen)	Chromosome counting by DNA FISH and membrane staining	Proximity to Zone 3 (central vein)	Polyploid hepatocytes enriched in Zone 2
Katsuda et al[46]	Rat	5 to 14 wk	Hepatocytes isolated from digested liver	FACS-isolation of diploid, tetraploid, and octaploid hepatocytes	Expression of zonal genes using bulk microarrays and sc-qRT-PCR	Polyploid hepatocytes enriched in Zone 1; diploid hepatocytes enriched in Zone 3
Richter et al[48]	Mouse	3 mo	Nuclei from frozen liver	FACS-isolation of diploid and tetraploid nuclei	Expression of zonal genes using snRNA-seq	Tetraploid nuclei enriched in Zone 3
Margall-Ducos et al[19]	Rat	10 to 25 d	Tissue sections (FFPE)	Cytokinesis failure as a marker of polyploid hepatocytes during postnatal development	Proximity to Zone 1 (PEPCK1) and Zone 3 (GS)	Polyploid hepatocytes equally distributed across all zones
Bou-Nader et al[10]	Human	24-86 y	Liver sections (FFPE)	Nuclear measurements (diameter, intensity)	Proximity to Zone 3 (GS)	Polyploid hepatocytes equally distributed across all zones
Yang et al[49]	Mouse	Embryonic day 17.5 to 2 mo	Hepatocytes isolated from digested liver	FACS isolation of diploid, tetraploid, and octaploid hepatocytes; nuclei isolated form sorted cells	Expression of zonal genes using scRNA-seq and in nuclei by snRNA-seq	Diploid hepatocytes enriched in Zone 1

Abbreviations: FACS, fluorescence-activated cell sorting; FFPE, formalin-fixed paraffin-embedded; FISH, fluorescence in situ hybridization; GS, glutamine synthetase; sc-qRT-PCR, single-cell quantitative reverse-transcription polymerase chain reaction; scRNA-seq, single-cell RNA sequencing; sn-RNAseq, single-nuclear RNA sequencing.

level using single-nuclear RNA-seq (snRNA-seq). Three-month-old mouse hepatocytes were lysed, and nuclei were sorted based on the DNA content. Sequencing revealed that nuclei with 4n content were enriched 1.3-fold in the expression of zone 3 genes.[48] It is difficult to determine how nuclear expression patterns translate to hepatic populations since 2n nuclei are found in diploid and binucleate tetraploid hepatocytes, and 4n nuclei are found in mononucleate tetraploids and binucleate octaploid hepatocytes. Fourth, evaluation of incomplete cytokinesis, a readout of polyploidization, among hepatocytes in the periportal and pericentral regions of developing rat livers revealed similar proportions of binucleate polyploid hepatocytes in both zone 1 and zone 3.[19] Next, Bou-Nader et al investigated the location of polyploids in human hepatic lobules using a nuclear-intensity staining approach and found that polypoid hepatocytes are equivalently distributed across all zones.[10] Finally, in a very recent study, Yang et al performed single-cell RNA-sequencing (scRNA-seq) and snRNA-seq on mice developing prenatally and postnatally.[49] They found preferential expression of periportal (zone 1) genes by diploid hepatocytes, suggesting the localization of diploids to the periportal region.

The abundance of contradictory data is striking and may be caused by differences in the tissues examined and techniques used to determine ploidy and zonation. The inherent variation in ploidy levels among species (mice, rats, and humans) introduces substantial variation in overall ploidy levels and possibly ploidy distribution. The age-dependent nature of ploidy, with cells exhibiting dynamic changes throughout postnatal development and adulthood, represents a significant source of variability. Using various methodological approaches, from nuclear intensity-based tissue assessments and DNA FISH to single-cell and single-nuclear RNA-seq approaches, introduces unique biases. Studying the spatial distribution is challenging, and it remains to be seen how diploid and polyploid hepatocytes are definitively arranged with the liver. It is clear, however, that diploids and polyploids reside in all zones, and further work is needed to fully understand the spatial differences reported by others and whether such changes affect liver function. To definitively resolve these issues, a multipronged approach may be necessary, including (1) spatial transcriptomics to discern the precise lobule location and cellular activity of hepatocytes; (2) identification of ploidy populations by nuclear fluorescence intensity and chromosome counting by DNA FISH; and (3) discrimination between mononucleate and binucleate hepatocytes with a membrane marker. This approach, allowing the simultaneous detection of the geographic location and ploidy levels of all hepatocytes, could be applied to multiple species during homeostasis and injury, facilitating a deeper understanding of the roles played by diploid and polyploid hepatocytes.

Gene Expression

The hepatic ploidy state could affect gene expression in multiple ways. It has been hypothesized that gene expression levels increase proportionally with ploidy.[17] Considering polyploid hepatocytes have increased DNA compared to diploids, it is conceivable that there is a dose-dependent effect on gene expression, leading to increased levels of gene expression and protein synthesis in polyploid cells. For example, a tetraploid cell has twice the amount of DNA as a diploid; thus, tetraploid hepatocytes may increase gene expression two-fold.[17] This dose-dependent effect has been observed in *Arabidopsis*, which contains diploid and tetraploid gametic cells, where the transcriptome of tetraploid cells is doubled compared to diploids.[50] It is unclear how gene dosage impacts transcriptomics in mammalian cells, but recent studies suggest that hepatocyte ploidy does not scale with gene expression. Yang et al performed scRNA-seq and snRNA-seq of mouse hepatocytes and found equivalent expression of mitochondrial and housekeeping genes by diploid and polyploid hepatocytes.[49] Similarly, using snRNA-seq, Richter et al found a 1.4-fold increase in the median gene number in 4n nuclei (compared to 2n nuclei), less than the predicted two-fold difference.[48] These findings suggest there are mechanisms to maintain gene dosage. One mechanism may be the silencing of supernumerary chromosomes in polyploid cells. Regulation of supernumerary chromosomes is known to occur in females, where the extra X chromosome is inactivated, equalizing X chromosome transcript levels between females (XX) and males (XY).[51] Intriguingly, in females with four X chromosomes, two are actively transcribed while two are silenced.[52] Whether polyploid hepatocytes regulate gene dosage through similar or distinct mechanisms remains unanswered.

Hepatic ploidy populations may exhibit varying gene expression patterns that confer specialized functions, but there is little consistency in the literature. In 2007, Lu et al compared gene expression of mouse diploid, tetraploid, and octaploid hepatocytes by microarray analysis and found gene expression to be broadly equivalent between ploidy populations.[53] This finding is supported by the results of bulk RNA-seq in a super-polyploid murine model (transgenic *Anln* knockdown), which showed no differentially expressed genes compared to control samples.[54] This contrasts with more recent findings from Katsuda et al.[46,47] Gene expression of diploid and polyploid hepatocytes in rat hepatocytes was interrogated by either bulk microarray analysis or sc-qRT-PCR using a custom panel of 47 genes. They showed that diploid hepatocytes were enriched with genes associated with the progenitor cell phenotype (*Axin2*, *Prom1*, and *Lgr5*). However, Richter et al identified equivalent expression of progenitor markers by diploid and polyploid nuclei.[48] Studies by Matsumoto et al investigated the expression of genes related to aging by bulk RNA-seq in sorted diploid and polyploid hepatocytes from both young and aged murine livers.[55] They identified hundreds of differentially expressed genes, with common trends in both age groups. Notably, genes related to immune response were consistently downregulated in polyploids compared to diploids, while genes associated with mitochondrial function were consistently upregulated in polyploid hepatocytes. This contrasts with observations by Yang et al showing equivalent expression of mitochondrial genes by diploid and polyploid hepatocytes.[49]

Conflicting data make it difficult to compile a comprehensive list of differentially expressed genes by diploid and polyploid hepatocytes. These discrepancies may arise from variations in tissue and experimental techniques, as discussed earlier. To determine a comprehensive and definitive list, a meta-analysis of the gene expression profiles reported by many groups is necessary to understand their contradictory findings and to identify common expression patterns. Overall, the data suggest that distinct functions may exist for ploidy populations, and additional research is necessary to determine where transcriptional or translational output varies between diploid and polyploid hepatocytes.

Ploidy and Regeneration

Ploidy and Proliferation

The liver has an incredible capacity for regeneration. Up to 90% of liver mass can be lost, and the organ can regenerate to its original size and function.[56] Several ideas regarding the functional significance of polyploidy in hepatocyte proliferation and regeneration exist. It was initially thought that polyploid hepatocytes were terminally differentiated with little capacity for proliferation, but this idea has been disproven.[17,57,58] Polyploid hepatocytes proliferate robustly after partial hepatectomy (PH), the surgical removal of up to two-thirds of liver mass.[59] Moreover, extensive proliferation by polyploid hepatocytes has been observed in vivo in liver repopulation studies using the FAH$^{-/-}$ model.[9,26,60,61]

Although diploid and polyploid hepatocytes are capable of proliferation, several studies have demonstrated an increased proliferative capacity for diploid hepatocytes. First, our group investigated repopulation capacity using competitive transplantation in the FAH$^{-/-}$ model. To prevent changes in ploidy associated with the ploidy conveyor (i.e., polyploidization, ploidy reversal, and re-polyploidization, ►**Fig. 1**), repopulation experiments were conducted with *Ef7*/*E2f8*-deficient hepatocytes.[26] *E2f7* and *E2f8* negatively regulate cytokinesis, and the deletion of *E2f7* and *E2f8* in the liver inhibits polyploidization, yielding livers where 80% of hepatocytes are diploid and 20% are polyploid.[24,26] When *E2f7*/*E2f8*-deficient hepatocytes (predominately diploid) and wild-type (WT) hepatocytes (predominately polyploid) were cotransplanted into FAH$^{-/-}$ mice, *E2f7*/*E2f8*-deficient hepatocytes consistently outperformed the WT hepatocytes, indicating a robust proliferative advantage for stable diploid hepatocytes. Next, to mitigate the potential off-target effect of *E2f7*/*E2f8* deletion, proliferation by ploidy populations was monitored during PH-induced liver regeneration in WT mice.[26] Hepatocytes were harvested over a time course after PH, and their ploidy and cell cycle status were determined. Diploid hepatocytes entered and completed the cell cycle faster than polyploid hepatocytes, which exhibited delayed cell cycle progression (►**Fig. 3A**). It is unclear why polyploid hepatocytes have restricted proliferation; one mechanism may involve PIDDosome activation, leading to p21 expression and cell cycle arrest (►**Fig. 2A**).[36]

Second, Heinke et al used retrospective radiocarbon (^{14}C) birth dating of cells to investigate physiological hepatocyte replacement in humans.[62] Hepatocyte nuclei from 29 human subjects aged 20 to 84 years were isolated by fluorescence-activated cell sorting (FACS) into ploidy populations, and genomic ^{14}C concentrations were determined using accelerator mass spectrometry. Mathematical modeling was applied—incorporating historic atmosphere, memory effects, and cell-cycle dynamics—to estimate hepatocyte age and renewal rates. Human hepatocytes were found to have ongoing and lifelong turnover that permits the liver to remain relatively young, at an average of just 3 years old. However, the age of individual hepatocytes is highly dependent on ploidy status. Diploid hepatocytes showed birth rates sevenfold higher than polyploids, suggesting that human hepatocyte replacement is highly dependent on diploid cells (►**Fig. 3B**).

Finally, Viswanathan et al studied the pathological regeneration of human hepatocytes in vitro.[63] Primary human hepatocytes were cultured and treated with acetaminophen to induce acute injury and compensatory regeneration. The ploidy state of treated hepatocytes was determined by evaluating nuclear size and intensity. Primary human hepatocytes under control conditions were distributed along 2n (22%), 4n (49%), and 8n+ (29%) ploidy states. In acetaminophen-treated hepatocytes, the 2n ploidy state doubled to 44%, whereas the 4n and 8n+ classes decreased to 39 and 16%, respectively. Human diploid hepatocytes significantly increased after acetaminophen toxicity, indicating that diploid hepatocytes may drive compensatory regeneration in humans (►**Fig. 3C**). Together, these studies show that diploid hepatocytes, compared to polyploids, have a strong proliferative advantage during homeostasis and liver regeneration. The proliferative capacity of diploid and polyploid hepatocytes needs to be evaluated in other contexts, such as chronic and acute injury models, where diploid hepatocytes are predicted to maintain a strong proliferative advantage.

Ploidy and Oncogenic Proliferation

Hepatocellular carcinoma (HCC) is the most common form of liver cancer and is associated with high mortality and morbidity globally.[64] There is substantial evidence that polyploidy is associated with heightened disease severity in many cancers, including pancreatic, cervical, and lung cancer.[15,65–67] It is tempting to propose that hepatic polyploidy might contribute to developing HCC; however, HCCs in both patient and rodent models are highly enriched in diploid hepatocytes.[68–70] Recent studies suggest that diploid hepatocytes drive HCC and that polyploidy protects the liver from tumorigenesis. Zhang et al showed that polyploid hepatocytes protect against liver cancer in the diethylnitrosamine (DEN) model.[71] Loss-of-heterozygosity of tumor suppressor genes, such as *Mll2*, *Arid1a*, or *Rb1*, enhances the transformation potential of diploid cells. However, additional chromosome sets in polyploid hepatocytes effectively provide "backup" copies of tumor suppressor genes to compensate for their loss of heterozygosity. Complementary studies by our lab found that *E2f7*/*E2f8*-deficient mice that are enriched with diploids form HCC better than controls, likely through accelerated proliferation by transformed diploid hepatocytes.[26] Research by Lin et al corroborates these findings.[54]

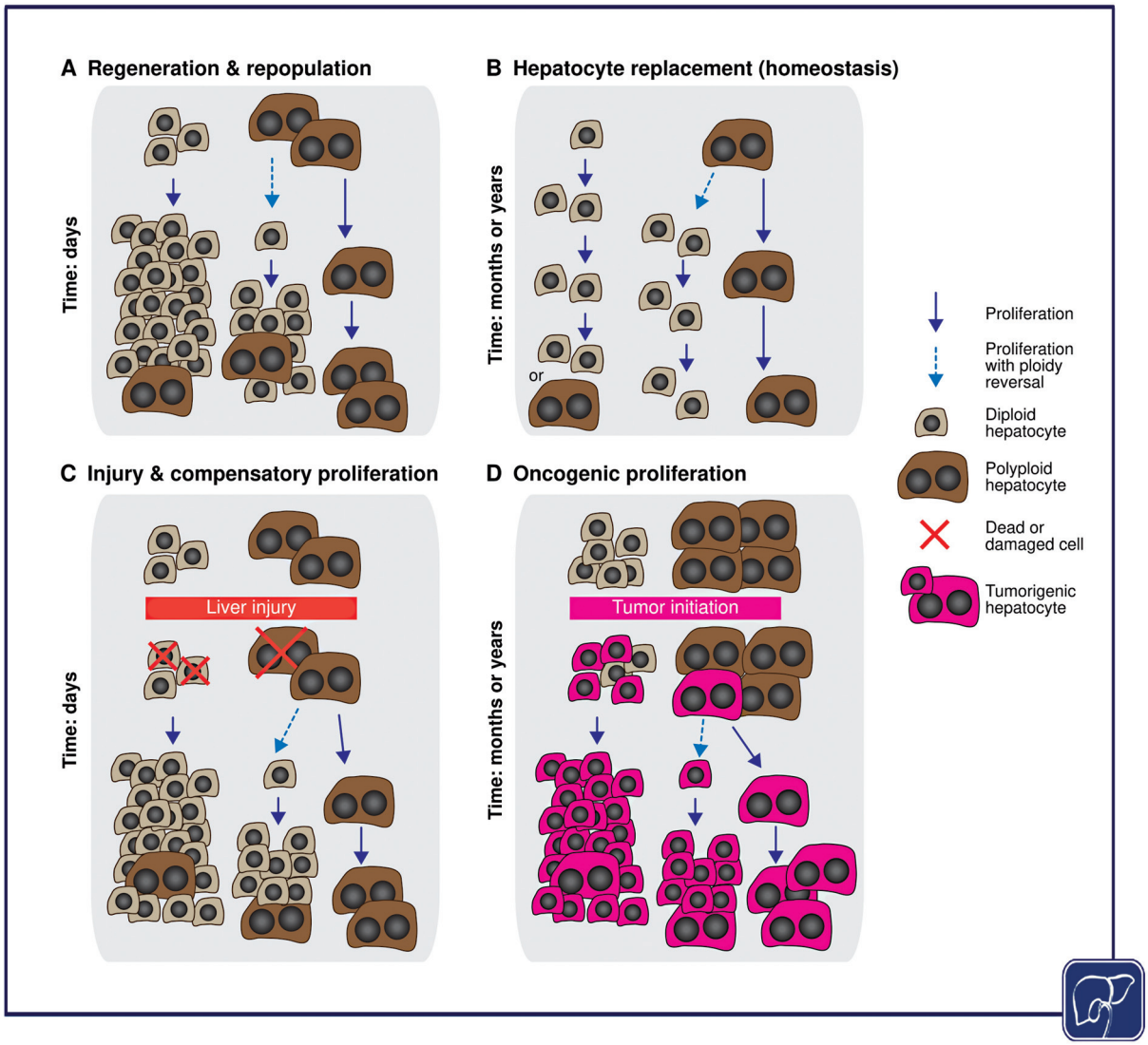


Fig. 3 Ploidy-influenced hepatocyte proliferation. (**A**) Diploid and polyploid hepatocytes contribute to liver regeneration and repopulation. In response to proliferative cues, diploid hepatocytes enter the cell cycle earlier and progress through the cell cycle faster than polyploid hepatocytes, driving early hepatocyte regeneration and repopulation. Polyploid hepatocytes enter the cell cycle slowly, possibly due to PIDDosome/p21-mediated arrest, and can generate polyploid daughters or diploid daughters via ploidy reversal. These diploid hepatocytes are capable of rapid proliferation and re-polyploidization. (**B**) Diploid and polyploid hepatocytes mediate hepatocyte replacement during normal aging (homeostasis). Hepatocyte turnover occurs primarily by diploids, arising from preexisting diploid hepatocytes or ploidy reversal by polyploid hepatocytes. (**C**) Liver injury involving hepatocyte death or damage is accompanied by compensatory proliferation. Diploid hepatocytes are the primary drivers of proliferation, although polyploid hepatocytes can proliferate with slower kinetics, generating polyploid hepatocytes or diploid hepatocytes (that can re-polyploidize). (**D**) Diploid and polyploid hepatocytes respond differently to tumorigenic insults. Polyploid hepatocytes, with additional copies of tumor suppressor genes, are relatively protected against tumor initiation (shown by one of four cells becoming tumorigenic), while diploid hepatocytes are sensitive to tumorigenesis (indicated by four of six cells becoming tumorigenic). Upon tumor initiation, tumorigenic diploid hepatocytes are capable of rapid proliferation, generating diploid and polyploid daughters. Tumorigenic polyploid hepatocytes also proliferate, albeit more slowly, generating polyploid daughters or diploid daughters (that can re-polyploidize).

Using superpolyploid mice (transgenic *Anln* knockdown), diploid-enriched mice (*E2f8* knockout), and controls, mice were injected with DEN and repeatedly dosed with carbon tetrachloride to induce liver regeneration. They found increased tumor formation in diploid-enriched livers compared to controls, while a decrease in tumor formation was observed in superpolyploid mice. Collectively, these data support the idea that diploid hepatocytes can drive liver cancer development, whereas polyploid hepatocytes protect the liver from tumorigenesis by providing extra copies of tumor suppressor genes and restricting hepatocyte proliferation (►**Fig. 3D**).

Although diploid HCCs are extensively described, polyploid HCCs have also been reported. For example, Bou-Nader and colleagues examined the ploidy distribution in human HCCs.[10] Thirty-three percent of HCCs were enriched with mononuclear polyploid hepatocytes, which correlated with poor differentiation, increased inflammation, p53 mutation, and increased expression of proliferation genes. Matsuura

et al very recently determined the ploidy status in human HCC samples by DNA FISH and detected polyploidy in 36% of HCCs.[72] Polyploid HCCs were particularly aggressive and associated with poor prognosis. Similarly, Lin et al identified microscopic foci of preneoplastic lesions surrounded by hyperpolyploid hepatocytes in mice treated with DEN. They suggest a possible link between the formation of hyperpolyploidy and the early stages of HCC.[73] Overall, these findings underscore the intricate role of polyploidy in HCC progression, which is likely influenced by various genetic factors and the dynamic nature of ploidy status (►**Fig. 3D**).

Ploidy Conveyor as a Mechanism of Ploidy Flexibility

During liver growth and regeneration, the liver undergoes remarkable and dynamic chromosomal changes. These changes are described by the ploidy conveyor model, which incorporates polyploidization, ploidy reversal, and re-polyploidization (►**Fig. 1**).[9,17] The ploidy conveyor was demonstrated by our group in 2010.[9] Fixed and live cell imaging was used to track cell cycling of diploid and polyploid hepatocytes in vitro. Diploid hepatocytes were observed to polyploidize, generating higher ploidy state daughters, while polyploid hepatocytes underwent ploidy reversal, generating lower ploidy daughters. Proliferation by mouse diploid and polyploid hepatocytes was also studied in vivo. Octaploid hepatocytes isolated by FACS from WT mice were transplanted into FAH$^{-/-}$ mice and underwent 500 to 10,000-fold proliferation to repopulate the liver. The ploidy distribution of donor-derived hepatocytes was assessed upon complete liver repopulation, revealing octaploid daughter hepatocytes, as expected, and lower ploidy daughters (tetraploid and diploid hepatocytes). Surprisingly, the donor-derived hepatocytes were found in ratios consistent with a "normal" ploidy distribution. Together, these studies demonstrate the dynamic nature of hepatic ploidy: proliferating diploids become polyploid, and proliferating polyploids can become diploid.

To determine the extent of dynamic ploidy shifts during liver injury and regeneration, Matsumoto et al developed lineage tracing systems.[20] Using Rosa-Conetti multicolor reporter mice, Cre recombination allowed the stochastic expression of a single fluorescent protein from each Rosa-Confetti allele (GFP, YFP, RFP, or CFP). In heterozygous Rosa-Confetti mice, diploid cells expressed one reporter and thus were monocolored, while polyploid cells could express multiple colors due to their additional sets of chromosomes. Cellular lineage was determined by tracking hepatocyte fluorescence. Bicolored tetraploid hepatocytes were separated by FACS, transplanted in FAH$^{-/-}$ mice, and allowed to repopulate the liver over 3 to 4 months. Analysis of repopulated livers showed that these hepatocytes underwent ploidy reversal. For example, liver repopulation by bicolored (YFP+ RFP+) tetraploid hepatocytes produced 35 to 50% monocolored daughters (YFP+ or RFP+), consistent with chromosome loss by a ploidy reversal mechanism. Moreover, 97% of their monocolored derivatives were polyploid. This suggests that YFP+ RFP+ hepatocytes underwent ploidy reversal by multipolar cell division to generate one YFP+ diploid daughter or one RFP+ diploid daughter, which, in turn, re-polyploidized to generate monocolored polyploid hepatocytes (i.e., YFP diploid polyploidized to form polyploid daughter cells expressing two YFP alleles). To determine the prevalence of dynamic ploidy changes associated with the ploidy conveyor, various chronic injury models (carbon tetrachloride, thioacetamide, and 3,5-diethoxycarbonyl-1,4-dihydrocollidine [DDC]) were applied. Compared to uninjured mice, there were half as many bicolored (YFP+ RFP+) hepatocytes but twice as many monocolored cells (YFP+ or RFP+), indicating a ploidy reversal mechanism in response to diverse types of liver injury. Together, these data indicate that ploidy reversal and subsequent re-polyploidization are common features of hepatocyte proliferation, seen in each injury model examined (►**Fig. 3A–C**).[20,74]

Although polyploidy can protect against liver cancer, polyploid hepatocytes are not immune to carcinogenesis (see *Ploidy and Oncogenic Proliferation*). Matsumoto and colleagues again used lineage tracing models to investigate the role of polyploidy and ploidy reversal during oncogenesis.[74,75] In response to induced tumorigenesis (nonalcoholic steatosis, FAH deficiency, and thioacetamide injury), multiple tumor lineages emerged, including bicolored tumors (derived from polyploid hepatocytes) and monocolored tumors (originally from ploidy reversal-derived diploids). Furthermore, a direct comparison of the tumorigenic potential of (1) ploidy reversal-competent hepatocytes and (2) experimentally induced incompetent hepatocytes revealed up to a seven-fold increase in tumor formation by hepatocytes capable of ploidy reversal. Thus, the data demonstrate that ploidy reversal and polyploid-derived diploid hepatocytes are critical intermediates in the development of hepatocyte-derived cancers. The ploidy conveyor may explain the emergence of polyploid HCCs.[10,72,73] It is possible that HCC initiation occurs in diploid hepatocytes lacking redundant tumor suppressor genes; as the disease advances, some undergo polyploidization (►**Fig. 3D**).[71] The significance of ploidy in HCC progression and its implications for prognosis warrants further investigation.

Concluding Remarks and a Model for Ploidy-Associated Hepatocyte Proliferation

The high degree of polyploidy in human and rodent livers suggests that diploid and polyploid hepatocytes perform specialized roles. Whether and how hepatocyte identity (e.g., zonation, gene expression) is influenced by ploidy is poorly appreciated, and contradictory observations in the liver must be resolved. Diploid and polyploid hepatocytes can proliferate and generate daughters with increased or decreased ploidy. The cycle of polyploidization, ploidy reversal, and re-polyploidization (i.e., the ploidy conveyor) is a specialized form of hepatic plasticity that occurs frequently during hepatocyte proliferation and injury (►**Fig. 4**). Hepatocytes with diploid nuclear content, whether de novo diploids or derived from polyploid hepatocytes, have an enhanced capacity to proliferate compared to hepatocytes with polyploid nuclear content. Thus, diploid hepatocytes drive robust proliferation, including

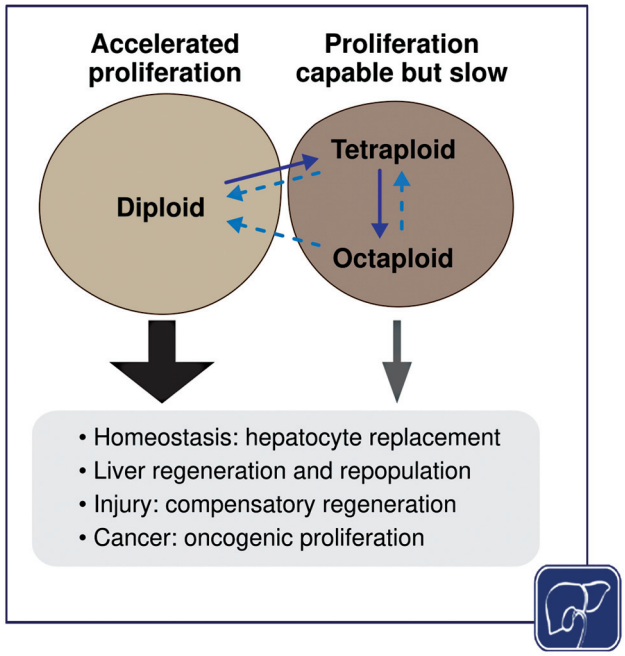


Fig. 4 Hepatocyte ploidy influences proliferative potential. Diploid, tetraploid, and octaploid hepatocytes can proliferate, but diploids are capable of accelerated proliferation. The slowly proliferating polyploids can undergo ploidy reversal to form rapidly proliferating diploid daughters. Diploid hepatocytes function as the primary drivers of hepatocyte replacement (homeostasis), liver regeneration and repopulation, compensatory proliferation after injury, and oncogenic proliferation in hepatocellular carcinoma. Solid purple lines indicate polyploidization; *dashed blue lines* mark ploidy reversal; and *black/gray arrows* indicate the relative contribution to proliferation.

physiological turnover, regeneration, and oncogenic proliferation.

Funding
This work was supported by grants to A.W.D. from the National Institutes of Health (National Institute of Diabetes and Digestive and Kidney Diseases; U.S. Department of Health and Human Services) (R01 DK103645) and the Commonwealth of Pennsylvania. S.R.W. was supported by the National Institute of Biomedical Imaging and Bioengineering. NIBIB training grant, T32 EB001026, entitled "Cellular Approaches to Tissue Engineering and Regeneration."

Conflict of Interest
None declared.

References

1 Patterson M, Swift SK. Residual diploidy in polyploid tissues: a cellular state with enhanced proliferative capacity for tissue regeneration? Stem Cells Dev 2019;28(23):1527–1539
2 Orr-Weaver TL. When bigger is better: the role of polyploidy in organogenesis. Trends Genet 2015;31(06):307–315
3 Neiman M, Beaton MJ, Hessen DO, Jeyasingh PD, Weider LJ. Endopolyploidy as a potential driver of animal ecology and evolution. Biol Rev Camb Philos Soc 2017;92(01):234–247
4 Vasudevan A, Schukken KM, Sausville EL, Girish V, Adebambo OA, Sheltzer JM. Aneuploidy as a promoter and suppressor of malignant growth. Nat Rev Cancer 2021;21(02):89–103
5 Weaver BA, Cleveland DW. Does aneuploidy cause cancer? Curr Opin Cell Biol 2006;18(06):658–667
6 Sheltzer JM, Amon A. The aneuploidy paradox: costs and benefits of an incorrect karyotype. Trends Genet 2011;27(11):446–453
7 Segal DJ, McCoy EE. Studies on Down's syndrome in tissue culture. I. Growth rates and protein contents of fibroblast cultures. J Cell Physiol 1974;83(01):85–90
8 Milne LS. The histology of liver tissue regeneration. J Pathol Bacteriol 1909;13(01):127–160
9 Duncan AW, Taylor MH, Hickey RD, et al. The ploidy conveyor of mature hepatocytes as a source of genetic variation. Nature 2010; 467(7316):707–710
10 Bou-Nader M, Caruso S, Donne R, et al. Polyploidy spectrum: a new marker in HCC classification. Gut 2020;69(02):355–364
11 Knouse KA, Wu J, Whittaker CA, Amon A. Single cell sequencing reveals low levels of aneuploidy across mammalian tissues. Proc Natl Acad Sci U S A 2014;111(37):13409–13414
12 Duncan AW, Hanlon Newell AE, Bi W, et al. Aneuploidy as a mechanism for stress-induced liver adaptation. J Clin Invest 2012; 122(09):3307–3315
13 Wilkinson PD, Alencastro F, Delgado ER, et al. Polyploid hepatocytes facilitate adaptation and regeneration to chronic liver injury. Am J Pathol 2019;189(06):1241–1255
14 Sladky VC, Eichin F, Reiberger T, Villunger A. Polyploidy control in hepatic health and disease. J Hepatol 2021;75(05):1177–1191
15 Donne R, Saroul-Aïnama M, Cordier P, Celton-Morizur S, Desdouets C. Polyploidy in liver development, homeostasis and disease. Nat Rev Gastroenterol Hepatol 2020;17(07):391–405
16 Gentric G, Desdouets C. Polyploidization in liver tissue. Am J Pathol 2014;184(02):322–331
17 Duncan AW. Aneuploidy, polyploidy and ploidy reversal in the liver. Semin Cell Dev Biol 2013;24(04):347–356
18 Guidotti JE, Brégerie O, Robert A, Debey P, Brechot C, Desdouets C. Liver cell polyploidization: a pivotal role for binuclear hepatocytes. J Biol Chem 2003;278(21):19095–19101
19 Margall-Ducos G, Celton-Morizur S, Couton D, Brégerie O, Desdouets C. Liver tetraploidization is controlled by a new process of incomplete cytokinesis. J Cell Sci 2007;120(Pt 20): 3633–3639
20 Matsumoto T, Wakefield L, Tarlow BD, Grompe M. In vivo lineage tracing of polyploid hepatocytes reveals extensive proliferation during liver regeneration. Cell Stem Cell 2020; 26(01):34–47.e3
21 Wilkinson PD, Duncan AW. Differential roles for diploid and polyploid hepatocytes in acute and chronic liver injury. Semin Liver Dis 2021;41(01):42–49
22 Celton-Morizur S, Merlen G, Couton D, Desdouets C. Polyploidy and liver proliferation: central role of insulin signaling. Cell Cycle 2010;9(03):460–466
23 Celton-Morizur S, Merlen G, Couton D, Margall-Ducos G, Desdouets C. The insulin/Akt pathway controls a specific cell division program that leads to generation of binucleated tetraploid liver cells in rodents. J Clin Invest 2009;119(07):1880–1887
24 Pandit SK, Westendorp B, Nantasanti S, et al. E2F8 is essential for polyploidization in mammalian cells. Nat Cell Biol 2012;14(11): 1181–1191
25 Chen HZ, Ouseph MM, Li J, et al. Canonical and atypical E2Fs regulate the mammalian endocycle. Nat Cell Biol 2012;14(11): 1192–1202
26 Wilkinson PD, Delgado ER, Alencastro F, et al. The polyploid state restricts hepatocyte proliferation and liver regeneration in mice. Hepatology 2019;69(03):1242–1258
27 Kent LN, Rakijas JB, Pandit SK, et al. E2f8 mediates tumor suppression in postnatal liver development. J Clin Invest 2016; 126(08):2955–2969

28 Hsu SH, Ghoshal K. MicroRNAs in liver health and disease. Curr Pathobiol Rep 2013;1(01):53–62
29 Hsu SH, Delgado ER, Otero PA, et al. MicroRNA-122 regulates polyploidization in the murine liver. Hepatology 2016;64(02): 599–615
30 Tinel A, Tschopp J. The PIDDosome, a protein complex implicated in activation of caspase-2 in response to genotoxic stress. Science 2004;304(5672):843–846
31 Fava LL, Schuler F, Sladky V, et al. The PIDDosome activates p53 in response to supernumerary centrosomes. Genes Dev 2017;31 (01):34–45
32 Sladky VC, Knapp K, Szabo TG, et al. PIDDosome-induced p53-dependent ploidy restriction facilitates hepatocarcinogenesis. EMBO Rep 2020;21(12):e50893
33 Liang CQ, Zhou DC, Peng WT, et al. FoxO3 restricts liver regeneration by suppressing the proliferation of hepatocytes. NPJ Regen Med 2022;7(01):33
34 Diril MK, Ratnacaram CK, Padmakumar VC, et al. Cyclin-dependent kinase 1 (Cdk1) is essential for cell division and suppression of DNA re-replication but not for liver regeneration. Proc Natl Acad Sci U S A 2012;109(10):3826–3831
35 Gentric G, Maillet V, Paradis V, et al. Oxidative stress promotes pathologic polyploidization in nonalcoholic fatty liver disease. J Clin Invest 2015;125(03):981–992
36 Sladky VC, Knapp K, Soratroi C, et al. E2F-family members engage the PIDDosome to limit hepatocyte ploidy in liver development and regeneration. Dev Cell 2020;52(03):335–349.e7
37 Hsu SH, Duncan AW. Pathological polyploidy in liver disease. Hepatology 2015;62(03):968–970
38 Colnot S, Perret C. Molecular pathology of liver diseases. Liver zonation Springer US 2011;5:7–16
39 Hoehme S, Brulport M, Bauer A, et al. Prediction and validation of cell alignment along microvessels as order principle to restore tissue architecture in liver regeneration. Proc Natl Acad Sci U S A 2010;107(23):10371–10376
40 Soto-Gutierrez A, Gough A, Vernetti LA, Taylor DL, Monga SP. Preclinical and clinical investigations of metabolic zonation in liver diseases: the potential of microphysiology systems. Exp Biol Med (Maywood) 2017;242(16):1605–1616
41 Hijmans BS, Grefhorst A, Oosterveer MH, Groen AK. Zonation of glucose and fatty acid metabolism in the liver: mechanism and metabolic consequences. Biochimie 2014;96:121–129
42 Wei Y, Wang YG, Jia Y, et al. Liver homeostasis is maintained by midlobular zone 2 hepatocytes. Science 2021;371(6532):eabb1625
43 Paris J, Henderson NC. Liver zonation, revisited. Hepatology 2022; 76(04):1219–1230
44 Jungermann K, Kietzmann T. Zonation of parenchymal and nonparenchymal metabolism in liver. Annu Rev Nutr 1996;16:179–203
45 Tanami S, Ben-Moshe S, Elkayam A, Mayo A, Bahar Halpern K, Itzkovitz S. Dynamic zonation of liver polyploidy. Cell Tissue Res 2017;368(02):405–410
46 Katsuda T, Hosaka K, Matsuzaki J, et al. Transcriptomic dissection of hepatocyte heterogeneity: linking ploidy, zonation, and stem/progenitor cell characteristics. Cell Mol Gastroenterol Hepatol 2020;9(01):161–183
47 Duncan AW. Single-cell and bulk transcriptome profiling reveals unique features of diploid and polyploid hepatocytes. Cell Mol Gastroenterol Hepatol 2020;9(01):193–194
48 Richter ML, Deligiannis IK, Yin K, et al. Single-nucleus RNA-seq2 reveals functional crosstalk between liver zonation and ploidy. Nat Commun 2021;12(01):4264
49 Yang L, Wang X, Zheng JX, et al. Determination of key events in mouse hepatocyte maturation at the single-cell level. Dev Cell 2023;58(19):1996–2010.e6
50 Song Q, Ando A, Jiang N, Ikeda Y, Chen ZJ. Single-cell RNA-seq analysis reveals ploidy-dependent and cell-specific transcriptome changes in Arabidopsis female gametophytes. Genome Biol 2020;21(01):178
51 Jacobson EC, Pandya-Jones A, Plath K. A lifelong duty: how Xist maintains the inactive X chromosome. Curr Opin Genet Dev 2022; 75:101927
52 Balaton BP, Dixon-McDougall T, Peeters SB, Brown CJ. The eXceptional nature of the X chromosome. Hum Mol Genet 2018;27(R2): R242–R249
53 Lu P, Prost S, Caldwell H, Tugwood JD, Betton GR, Harrison DJ. Microarray analysis of gene expression of mouse hepatocytes of different ploidy. Mamm Genome 2007;18(09):617–626
54 Lin YH, Zhang S, Zhu M, et al. Mice with increased numbers of polyploid hepatocytes maintain regenerative capacity but develop fewer hepatocellular carcinomas following chronic liver injury. Gastroenterology 2020;158(06):1698–1712.e14
55 Matsumoto T, Wakefield L, Grompe M. The significance of polyploid hepatocytes during aging process. Cell Mol Gastroenterol Hepatol 2021;11(05):1347–1349
56 Gaub J, Iversen J. Rat liver regeneration after 90% partial hepatectomy. Hepatology 1984;4(05):902–904
57 Gupta S. Hepatic polyploidy and liver growth control. Semin Cancer Biol 2000;10(03):161–171
58 Fausto N, Campbell JS. The role of hepatocytes and oval cells in liver regeneration and repopulation. Mech Dev 2003;120(01): 117–130
59 Miyaoka Y, Ebato K, Kato H, Arakawa S, Shimizu S, Miyajima A. Hypertrophy and unconventional cell division of hepatocytes underlie liver regeneration. Curr Biol 2012;22(13):1166–1175
60 Overturf K, Al-Dhalimy M, Finegold M, Grompe M. The repopulation potential of hepatocyte populations differing in size and prior mitotic expansion. Am J Pathol 1999;155(06):2135–2143
61 Weglarz TC, Degen JL, Sandgren EP. Hepatocyte transplantation into diseased mouse liver. Kinetics of parenchymal repopulation and identification of the proliferative capacity of tetraploid and octaploid hepatocytes. Am J Pathol 2000;157(06): 1963–1974
62 Heinke P, Rost F, Rode J, et al. Diploid hepatocytes drive physiological liver renewal in adult humans. Cell Syst 2022;13(06): 499–507.e12
63 Viswanathan P, Sharma Y, Gupta P, Gupta S. Replicative stress and alterations in cell cycle checkpoint controls following acetaminophen hepatotoxicity restrict liver regeneration. Cell Prolif 2018; 51(03):e12445
64 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66(01):7–30
65 Sato N, Mizumoto K, Nakamura M, et al. Centrosome abnormalities in pancreatic ductal carcinoma. Clin Cancer Res 1999;5(05): 963–970
66 Olaharski AJ, Sotelo R, Solorza-Luna G, et al. Tetraploidy and chromosomal instability are early events during cervical carcinogenesis. Carcinogenesis 2006;27(02):337–343
67 Lothschütz D, Jennewein M, Pahl S, et al. Polyploidization and centrosome hyperamplification in inflammatory bronchi. Inflamm Res 2002;51(08):416–422
68 Saeter G, Schwarze PE, Nesland JM, Juul N, Pettersen EO, Seglen PO. The polyploidizing growth pattern of normal rat liver is replaced by divisional, diploid growth in hepatocellular nodules and carcinomas. Carcinogenesis 1988;9(06):939–945
69 Schwarze PE, Saeter G, Armstrong D, et al. Diploid growth pattern of hepatocellular tumours induced by various carcinogenic treatments. Carcinogenesis 1991;12(02):325–327
70 Nagasue N, Kohno H, Chang YC, et al. DNA ploidy pattern in synchronous and metachronous hepatocellular carcinomas. J Hepatol 1992;16(1–2):208–214
71 Zhang S, Zhou K, Luo X, et al. The polyploid state plays a tumor-suppressive role in the liver. Dev Cell 2018;44(04):447–459.e5

72 Matsuura T, Ueda Y, Harada Y, et al. Histological diagnosis of polyploidy discriminates an aggressive subset of hepatocellular carcinomas with poor prognosis. Br J Cancer 2023;129(08):1251–1260
73 Lin H, Huang YS, Fustin JM, et al. Hyperpolyploidization of hepatocyte initiates preneoplastic lesion formation in the liver. Nat Commun 2021;12(01):645
74 Matsumoto T. Implications of polyploidy and ploidy alterations in hepatocytes in liver injuries and cancers. Int J Mol Sci 2022;23 (16):9409
75 Matsumoto T, Wakefield L, Peters A, Peto M, Spellman P, Grompe M. Proliferative polyploid cells give rise to tumors via ploidy reduction. Nat Commun 2021;12(01):646