The American Journal of Pathology, Vol. 184, No. 2, February 2014

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The American Journal of PATHOLOGY

ajp.amjpathol.org

Liver Pathobiology Theme Issue

# REVIEW

# Polyploidization in Liver Tissue

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**CME Accreditation Statement:** This activity ("ASIP 2014 AJP CME Program in Pathogenesis") has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the American Society for Clinical Pathology (ASCP) and the American Society for Investigative Pathology (ASIP). ASCP is accredited by the ACCME to provide continuing medical education for physicians.

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**CME Disclosures:** The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.

Accepted for publication June 20, 2013.

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Polyploidy (alias whole genome amplification) refers to organisms containing more than two basic sets of chromosomes. Polyploidy was first observed in plants more than a century ago, and it is known that such processes occur in many eukaryotes under a variety of circumstances. In mammals, the development of polyploid cells can contribute to tissue differentiation and, therefore, possibly a gain of function; alternately, it can be associated with development of disease, such as cancer. Polyploidy can occur because of cell fusion or abnormal cell division (endoreplication, mitotic slippage, or cytokinesis failure). Polyploidy is a common characteristic of the mammalian liver. Polyploidization occurs mainly during liver development, but also in adults with increasing age or because of cellular stress (eg, surgical resection, toxic exposure, or viral infections). This review will explore the mechanisms that lead to the development of polyploid cells, our current state of understanding of how polyploidization is regulated during liver growth, and its consequence on liver function. *(Am J Pathol 2014, 184: 322–331; http://dx.doi.org/10.1016/j.ajpath.2013.06.035)*

Eukaryotic organisms usually contain two complete haploid sets of homologous chromosomes (diploid, 2n), the diploid state being a standard for sexually reproducing species. Polyploidy refers to the presence of additional sets of chromosomes (eg, 4n or 8n). These additional sets may originate from a single species (autopolyploidy) or from different, generally closely related, species (allopolyploidy). Polyploidy is most common among plants, particularly angiosperms.[1] These polyploid species commonly arise from unreduced gametes by nondisjunction of chromosomes in the germ line. Polyploidy is likely to modify plant morphological, phenological, physiological, and/or ecological characteristics and, thus, generates individuals that can flourish in novel habitats and fluctuating environments, or outcompete progenitor species.[2] Polyploidy is also tolerated in animals[3] (eg, fish and amphibians can form interspecific hybrids of varying ploidy). In contrast, in birds and mammals, whole-organism polyploidy is rare, and usually fatal, with polyploids dying early in their development. In humans, triploid and tetraploid fetuses are usually aborted or die soon after birth because of multiple internal and external malformations.[4,5] However, the supposed impossibility of polyploidy in mammals has been disproved by the discovery of tetraploidy in both red and golden viscacha rats.[6]

Polyploid cells can, however, be found at relatively high frequency in much mammalian tissue (Figure 1). In physiological conditions, the conversion from diploidy to polyploidy is a part of development and differentiation programs.[7] Polyploidization is seen, for example, in skeletal muscle, heart, placenta, liver, brain, and blood cells. In certain tissue, the

Supported by an Institut National de la Santé et de la Recherche Médicale (INSERM) grant, the Agence Nationale de la Recherche (ANR2010-BLAN112302) grant, the Fondation ARC pour la Recherche sur le Cancer grant (SFI20111203568), the Association Française pour l'Etude du Foie grant, and the Laboratoire Janssen Cilag grant. G.G. is a recipient of DIM Région Ile de France Cardiovasculaire-Obésité-Rein-Diabète.

This article is part of a review series on liver pathobiology.


http://dx.doi.org/10.1016/j.ajpath.2013.06.035

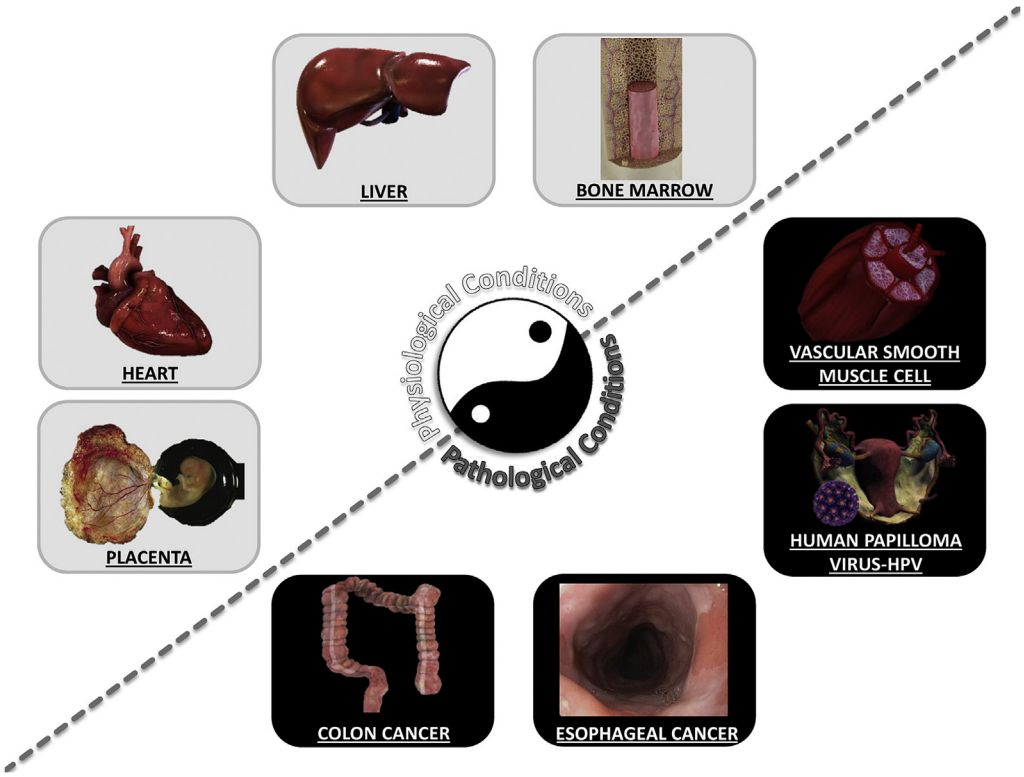


**Figure 1** Polyploidy during physiological and pathological processes. Polyploid cells are generated during physiological processes, such as embryogenesis (placenta-trophoblast giant cells), postnatal development (heart-cardiomyocytes or liver-hepatocytes), and terminal differentiation (bone marrow—megakaryocytes); and also during pathological processes, such as hypertension (vascular smooth muscle cells), virus infection (human papilloma virus), and tumorigenesis (esophageal and colon cancers).

genesis of polyploid cells is also linked to a variety of cellular stressors (eg, mechanical or metabolic stress). This has been well documented for uterine smooth muscle during pregnancy,[8] heart muscle and vascular smooth muscle cells during aging and hypertension,[9,10] and thyroid cells in hyperthyroidism.[11] Finally, unscheduled tetraploidy is observed in human cancers.[12] Tetraploidy constitutes a metastable intermediate between healthy diploidy and neoplastic aneuploidy. Tetraploidization has been observed, for example, in the early stages of colon cancer, breast cancer, Barrett's esophagus, and cervical cancer. In short, in mammals, the genesis of polyploid cells in a tissue can either contribute to differentiation and possibly a gain of function or, on the other hand, be associated with the development of diseases, such as cancer. Hepatic polyploidy is a characteristic feature of mammalian liver and was discovered a long time ago. Polyploidy is linked to postnatal development and varies in adults in response to various stimuli and aggression. In this review, we will explore the mechanisms leading to polyploidization in mammals and discuss how this process takes place in liver parenchyma and, thus, the functional consequences for liver proliferation and function.

## Causes of Whole Chromosome Duplication

How does a diploid tissue become polyploid? In a physiological or pathological context, there are several mechanisms that promote the genesis of polyploid cells: i) cell fusion, ii) endoreplication, iii) mitotic slippage, and iv) cytokinesis failure. The various mechanisms are described later.

### Cell Fusion

Cell-cell fusion is the only mechanism that leads to the genesis of a polyploid cell without the involvement of the cell cycle (Figure 2) and allows the formation of tissues and also produces specific functions. This mechanism plays a contradictory role, particularly involved in physiological development (eg, muscle fiber formation), but also in pathological events (viral infection), either linked to cancer development or unrelated.[13,14] During the physiological process, cell fusion is generally associated with terminally differentiated multinuclear cell formation.[15] Cell fusion has been well conserved through evolution, from yeast mating pairs to human muscle development. Myoblast fusion during embryogenesis has been extensively described thanks to *in vivo* studies in *Drosophila*. Cell fusion involves two different cell types: muscle founder cells and fusion-competent myoblasts. The mechanism can be divided into several stages, beginning with an attraction-recognition-adhesion stage,[16] dependent on complexes containing transmembrane proteins (with an immunoglobulin domain), such as Dumbfounded/Roughest and Sticks/Stones, expressed by the two cell types. These complexes allow actin

cytoskeleton rearrangements with migration and filopodia formation. The following stage involves actin accumulation at the fusion site to form a ring structure, fusion-restricted myogenic-adhesive structure. The last stage is associated with membrane breakdown and the removal of fusion machinery to allow a next round of fusion until final muscle fiber size is reached. Interestingly, experiments on cell plasticity have revealed that stem cells can fuse with mature cells in different tissues after transplantation (described later).

Cell-to-cell fusion is involved in pathological processes after viral infection. Cell fusion was first observed in virus infection in mouse cell culture infected with Sendai virus. Since this discovery, numerous viruses have been characterized to display fusogenic activity [eg, human papilloma virus (HPV), Epstein-Barr virus, Kaposi's sarcoma virus, hepatitis B virus, and hepatitis C virus],[17] in which cell-to-cell fusion contributes to cancer pathogenesis. Cervical cancer progression is strongly associated with HPV (HPV-16 and HPV-18) infection, detected in nearly all cases of cervical cancers. Expression of the oncoprotein HPV-16 E5 is sufficient for the formation of binucleated cells, a common characteristic of precancerous cervical lesions.[18] In association, HPV-16 E6 and E7 have been shown to inhibit the function of p53 and pRb and promote chromosomal instability.[17]

## Endoreplication

Polyploid cells can be generated by a mechanism that uncouples DNA replication from cell division (alias endoreplication).[19] Endoreplication can occur through endocycling, in which periods of S and G phases alternate with no cell division, or through endomitosis, which displays features of mitosis but lacks cytokinesis (Figure 2). In both cases, mononuclear progeny are generated (Figure 2).

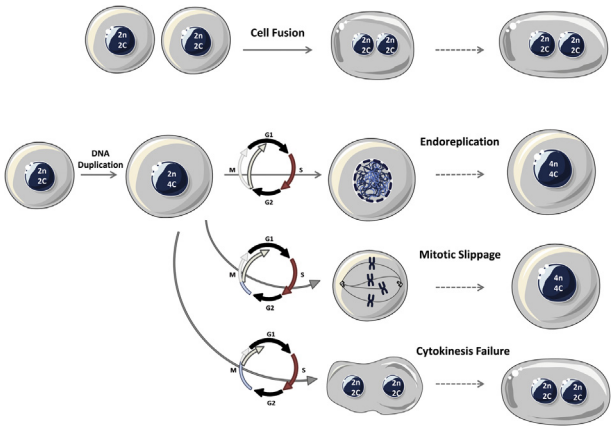


**Figure 2** Mechanisms leading to the genesis of tetraploid cells. Tetraploid cells can be generated by cell-to-cell fusion, or by abortive cell cycles after DNA replication: endoreplication, mitotic slippage, or cytokinesis failure. Although cell fusion and cytokinesis failure produce binuclear progeny, endoreplication and mitotic slippage result in a mononuclear cell (no karyokinesis). c, chromatid number; n, chromosome number.

Endoreplication occurs in the life cycle of protozoa, plants, flies, and mammals and often produces terminally differentiated cells that remain physiologically active but non-proliferating. This process has been extensively studied in *Drosophila*, in which cells in most larval tissues, and in many adult tissues, switch to endoreplication cycles. The master regulator is cyclin E (CycE) protein, whose periodic production activates CDK2 and promotes entry into the endoreplication cycle. The periodic transcription of CycE is controlled by the dimeric transcription factor, E2F1-DP, a central component of the endoreplication transcriptional oscillator.[20] The principal target of cyclin E/Cdk2 is Fzr/Cdh1, a positive regulator of the anaphase-promoting complex/cyclosome (APC/C).[21] The oscillatory activity of cyclin 2/CycE generates anti-parallel oscillations of APC/C-Fzr that are essential for both entry and progression through successive endoreplication cycles. Developmentally, endoreplication is rare in mammals, although one well-characterized example is differentiation of trophoblast stem cells into trophoblast giant cells. These cells are the first cell type to terminally differentiate during embryogenesis and are of vital importance for implantation and modulation of postimplantation placentation. The DNA content of TG cells generally ranges from 8N to 64N. In trophoblast stem cells, the transition from mitotic cell cycles to endoreplication cycles occurs when fibroblast growth factor 4 deprivation induces expression of the Cdk inhibitor, p57. Expression of p57 inhibits CDK1, allowing prereplicative assembly. Subsequent endoreplication cycles are driven by oscillation of cyclin E/Cdk2 and p57 activity.[22,23] Interestingly, recent data have highlighted the crucial role of E2F transcription factors in regulating mammalian polyploidy in TG cells and hepatocytes.

Numerous studies have shown a link between persistent DNA damage response and endoreplication cycles that induce polyploidy.[7,24] More precisely, double-stranded DNA breaks, accumulation of single-stranded DNA breaks, and telomere shortening induce a $G_2$/M block assumed by ataxia telangiectasia mutated/ataxia telangiectasia-mutated and Rad3-related, DNA damage sensor kinases. Irreparable damage can induce an irreversible cell cycle arrest, leading to cell death or a senescent state. However, in a $p53^{-/-}$ context, cells escape this arrest and undergo polyploidization by repeating endoreplication cycles. In different systems, a clear link between amplification of genomic instabilities and the endoreplication process has been demonstrated.[24] de Lange and collaborators showed that persistent telomere damage in a $p53^{-/-}$ context drives tetraploidization and enhances tumorigenic transformation of mouse fibroblast cells.[7,25]

## Mitotic Slippage

Polyploid cells can be formed after a prolonged arrest in metaphase because of the activation of the spindle assembly checkpoint (SAC) (Figure 2). The SAC prevents an advance to anaphase until all of the chromosomes are properly attached to

kinetochores. In the presence of unattached or weakly attached kinetochores, an inhibitory complex (Mad2, BubR1/Mad3, or Bub3) sequesters Cdc20, the APC/C co-activator. Once all of the chromosomes are attached, the SAC is silenced, leading to APC/C activation. In this context, cyclin B1 and securin are degraded, promoting anaphase onset and mitotic exit. Failure to satisfy the SAC can lead cells to slip out of the arrest by a phenomenon called mitotic slippage. Slippage is thought to occur by gradual proteolysis of cyclin B1, which continues slowly, even when the SAC is active.[26] Cells that are derived from mitotic slippage will contain a single tetraploid nucleus (Figure 2). Mitotic slippage has been observed in cells after prolonged mitotic arrest in response to spindle toxins.[27] Drugs that target microtubule dynamics (eg, taxanes, vinca alkaloids, and epothilones) are active against a broad range of cancers, activating the SAC. After many hours of SAC induction, cancer cells either die during mitosis (mitotic catastrophe) or exit mitosis by slippage into a tetraploid $G_1$ state, from which they die, arrest in $G_1$, or initiate a new round of the cell cycle. Mitotic slippage has been reported in adenomatous polyposis coli (APC)−deficient cells. APC mutation is the most frequent mutation found in human colorectal tumors, contributing to the genetic instability required for the progression from benign polyp to aggressive carcinoma. APC associates with mitotic spindle microtubules, most notably at the plus ends of the microtubules, which interact with kinetochores.[28] Both RNA interference-mediated depletion in cultured cells and conditional knockout of APC *in vivo* induce mitotic slippage linked to the genesis of a tetraploid contingent.[29] Caldwell and Kaplan[28] have also demonstrated that APC mutations lead to cytokinesis failure (described later).

## Cytokinesis Failure

Cytokinesis is the final step in cell division, leading to the physical separation of the sister cells. In mammals, it relies on complex and coordinated cell shape changes associated with membrane and cytoskeleton rearrangements.[30] Cytokinesis failure can arise through defects in any of the four stages of the process: i) positioning of the division plane, ii) ingression of the cleavage furrow, iii) formation of the midbody, and iv) abscission.

Cytokinesis failure is likely the mechanism underlying many of the ploidy changes that are observed in human tumors.[7,31] Tetraploid cells generated through cytokinesis failure (Figure 2) are relatively unstable compared with their diploid counterparts and frequently become aneuploid on continued cell division. Successful cytokinesis requires the interplay of several factors related to the cytoskeleton, chromosome, cell cycle, lipid raft, vesicle, and membrane trafficking factors.[32] Inactivation or hyperactivation of many of these factors has been shown to induce cytokinesis failure, leading to genesis of tetraploid binucleated cells.[33] As an example, amplification of Aurora A, Aurora B, and polo-like kinase 1 generates polyploidy via cytokinesis failure, with these proteins being overexpressed in different human tumors. Cytokinesis defects can also occur as the result of chromosome missegregation (lagging or bridge chromosomes). In fact, 1% of somatic cell divisions produce missegregation.[34] This occurs because of dysfunctional telomeres, DNA double-stranded breaks, or misregulated chromosome cohesion or decatenation.[35] During cytokinesis, chromatin trapped during furrow ingression either causes the cell to undergo apoptosis or inhibits cell abscission, producing tetraploid progenies in the latter case. Interestingly, a recent study demonstrated that Aurora B mediates an abscission checkpoint and delays completion of division to protect against tetraploidization by furrow regression.[36]

Finally, several diseases are associated with cytokinesis failure and polyploidization. A recent review by Lacroix and Maddox[37] clearly exposed these data. Fanconi anemia is one such genomic instability disorder characterized by bone marrow failure and predisposition to cancer. In an elegant study, Vinciguerra et al[38] demonstrated that cells deficient in Fanconi anemia proteins display high rates of polyploidy as a result of cytokinesis failure.

Remarkably, the cytokinesis failure process can also be a programmed stage in normal development, producing differentiated polyploid progenies.[7] An interesting case of ploidization is the cardiac muscle cells. During prenatal development, the development of the heart results in a proliferation of cardiomyocytes (hyperplasia).[39] After birth, ventricular cardiomyocytes respond to an amplification of blood flow with an adaptive increase in volume (hypertrophy). This transition from hyperplasia to hypertrophy is clearly associated with tetraploidization.[40] Different mechanisms have been proposed for cardiomyocyte tetraploidization. Cyclin $G_1$ has been identified as an important component of the molecular machinery controlling this process. Directed expression of cyclin $G_1$ in neonatal cardiomyocytes promotes $G_1$/S cell cycle transition but inhibits cytokinesis, thereby promoting cardiomyocyte tetraploidy.[41] Also, defective localization of anillin in the midbody region causes abnormal contractile ring formation.[42] Likewise, a drastic postnatal reduction of RhoA, Cdc42, Rac1, Rho-associated kinase I and II, and *p*-cofilin, coupled with the formation of the actomyosin ring, could account for defects in cytokinesis.[43] A second example of polyploidization involving cytokinesis failure is megakaryocytes (MKs).[44] In MK cells, anaphase and telophase can occur, but cytokinesis fails because of a regression of the cleavage furrow; polyploidization up to 128n can occur. Numerous studies have shown that polyploidy in MKs is regulated by a series of signaling pathways.[44] MK cell lines and primary MKs show decreased levels of cyclin B1 in polyploid MKs.[45] RhoA accumulation is also a key target in MK polyploidization. Indeed, down-regulation of the guanidine exchange factor, ECT2, prevents RhoA activation and cleavage furrow ingression in MK cells.[46] Finally, cyclin E1 plays a key role in promoting megakaryocyte entry into S phase and, hence, an increase in the number of cycling cells, augmenting polyploidization.[47]

## Polyploidy in the Liver

The liver is an essential organ that performs multiple functions essential for the maintenance of homeostasis. It has a central role in the metabolism, synthesis, storage, and redistribution of nutrients, carbohydrates, fats, and vitamins. The liver is also the main detoxifying organ of the body, and removes waste and xenobiotics by metabolic conversion and biliary excretion. Although the liver is made up of various types of cells, hepatocytes account for 78% of liver volume and 70% of all liver cells[48]; the liver function is mainly dependent on hepatocytes. Interestingly, hepatocytes have unique functions according to their position along the portocentral axis of the liver-cell plate (alias metabolic zonation).[49] Hepatocytes are quiescent and highly differentiated polyploid cells with an average life span of 200 to 300 days. During postnatal growth, the liver parenchyma undergoes dramatic changes characterized by gradual polyploidization during which hepatocytes of several ploidy classes emerge.[50–52] This process mainly generates tetraploid and octoploid hepatocytes with one nucleus (mononuclear; eg, 4n or 8n) or two nuclei (binuclear; eg, 2 × 2n or 2 × 4n). In fact, during liver development, most hepatocytes undergo a conventional cell cycle that leads to the genesis of two diploid hepatocytes (Figure 3A). However, some of them fail cytokinesis, leading to the genesis of binuclear tetraploid hepatocytes. Mononuclear progenies are generated after binuclear hepatocyte division (Figure 3A). When these cells divide, during mitosis, centrosomes cluster to form bipolar spindles, leading to the genesis of mononuclear hepatocytes,[52] followed by polyploidization to generate tetraploids and octoploids with one or two nuclei (Figure 3A). Remarkably, during postnatal development and during the organism's life, hepatocytes can also reduce their ploidy by a process call ploidy reversal (Figure 3C).[53] Octoploid and tetraploid hepatocytes undergo reductive mitosis. In these cells, multipolar spindles (in most cases, transient) are formed and, consequently, lagging chromosomes are observed in anaphase; they lead to the genesis of aneuploid daughter cells that contain a near-tetraploid and near-diploid chromosome number.[55]

The degree of liver polyploidization varies between mammals.[56] In rodents, hepatocyte polyploidization starts at the time of weaning, related to commencement of feeding,[57–59] and continues throughout development and aging. In adult rodents, the degree of polyploidy is high, up to 85% in C57Bl mice and Wistar rats.[53,60] In humans, polyploid hepatocytes appear at an early age, but their

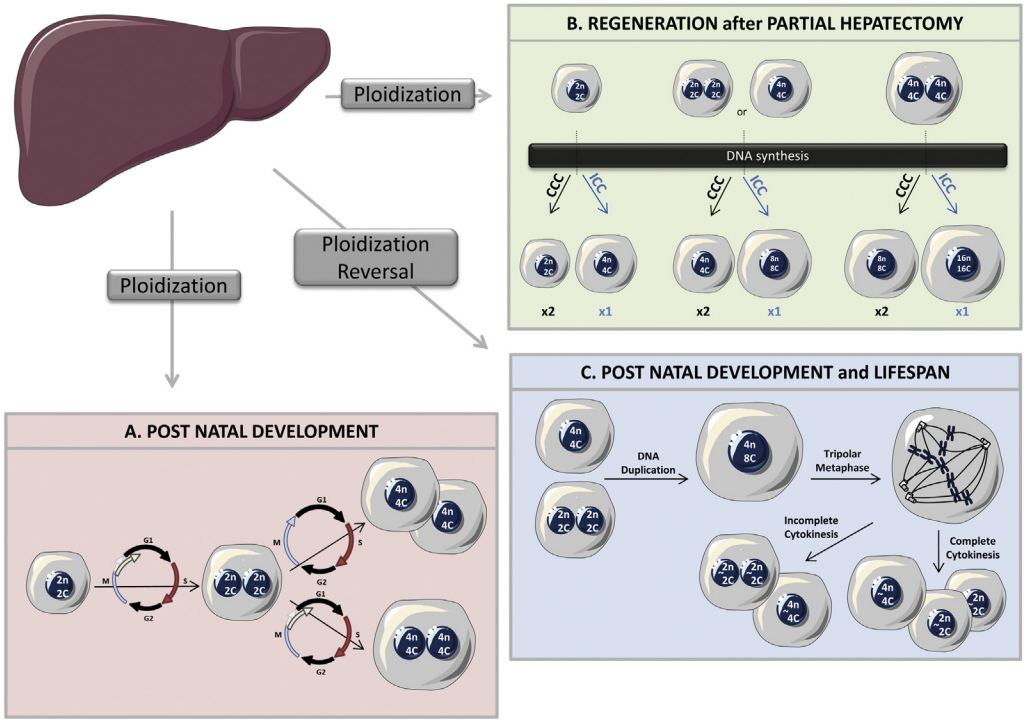


**Figure 3** Liver parenchyma and hepatocyte ploidy. During postnatal development, polyploidization takes place. Diploid hepatocytes can mostly go through a cell cycle with karyokinesis, but failure of cytokinesis results in a binuclear tetraploid hepatocyte that reenters the cell cycle and either undergoes cytokinesis, leading to the genesis of mononuclear tetraploid hepatocytes, or fails again, leading to the genesis of a binuclear octoploid hepatocyte (**A**). During liver regeneration after partial hepatectomy, diploid and polyploid contingents enter the cell cycle; some undergo cell division (completed cell cycle), generating two daughter cells, and some escape mitosis (incompleted cell cycle), generating only one daughter cell.[54] These processes increase the number of mononuclear hepatocytes, with the disappearance of the binuclear fraction. **B**: During postnatal development, and during the whole lifespan, polyploid hepatocytes can generate reduced polyploid progenies as the result of multipolar mitosis. As an example, tetraploid hepatocytes after the formation of tripolar metaphase will either complete cytokinesis (genesis of near-tetraploid and near-diploid progenies) or incomplete cytokinesis (genesis of near-tetraploid mononuclear and binuclear progenies)[53] (**C**). c, chromatid number; n, chromosome number.

number only increases slowly until the age of 50 years, when polyploidization intensifies.[61] In adults, approximately 30% to 40% of hepatocytes are polyploid.[62] Increased cell size is the most obvious consequence of an increase in ploidy, correlation between DNA content, and cell volume having been well described during evolution in distant eukaryotes.[31] Different studies in human and mouse liver cells have demonstrated that the volume of hepatocyte nuclei approximately doubles with the doubling of DNA content.[52,63,64] In contrast, there is no significant difference in the volume of polyploid hepatocytes containing one or two nuclei (eg, tetraploid contingent: binuclear 2 × 2n and mononuclear 4n).[64]

Hepatocytes in adult rodents and humans have a long lifespan and rarely divide under normal conditions. However, these cells retain a remarkable ability to proliferate in response to massive cellular injury from surgical resection, toxic exposure, or viral infection. Under all these circumstances, the liver polyploidy profile is modified. Liver regeneration induced by a two thirds partial hepatectomy (PH) in rats and mice is associated with a depletion of diploid and binuclear polyploids and accumulation of mononuclear polyploid hepatocytes.[51,65–67] A recent study by Miyajima and collaborators analyzed ploidy modification after 30% and 70% liver resection by using genetic fate mapping and a high-throughput imaging system of individual hepatocytes.[54] During liver regeneration after 70% PH, all hepatocytes (mononuclear and binuclear) entered the cell cycle. However, not all hepatocytes completed cell division, suggesting that an incomplete cell cycle occurs after PH. The authors suggested that only a few hepatocytes entered the M phase (Figure 3B). More important, if the binuclear contingent progresses though mitosis, it undergoes cytokinesis, generating two mononuclear daughter hepatocytes (Figure 3B). Remarkably, regeneration after 30% PH is achieved solely by cell hypertrophy without proliferation. In this case, the ploidy of hepatocytes is not modified. In adults, hepatic polyploidy is also clearly modified by cellular stress, such as metabolic overload (iron or copper),[68–70] telomere dysfunction,[71] and chronic hepatitis B and C infection.[62,72] Oxidative injury may play a role in liver polyploidy. Gupta and colleagues[73] show that hepatic polyploidy after two-thirds PH was associated with oxidative DNA injury. Interestingly, in transgenic mice overexpressing antioxidant enzymes, PH-induced polyploidization is decreased.[74] Similarly, treatment with aminoguanidine, which attenuates oxidative stress, decreased polyploidy.[75] In conclusion, extensive correlation exists between polyploidization and a variety of cellular stresses in the adult liver; however, the mechanisms leading to the genesis of polyploid hepatocytes and the consequence on liver parenchyma function are still unknown.

## How Hepatocytes Become Polyploid

Polyploidization mainly occurs through cytokinesis failure. During postnatal growth, some hepatocytes accomplish karyokinesis but do not form a contractile ring.[76,77] During this process, the actin cytoskeleton is not reorganized into the division plane in anaphase-telophase transition, impairing cell elongation. In concert, microtubules fail to contact the cortex and, therefore, molecular signals, essential for furrow induction (eg, Aurora B and polo-like kinase 1), are not delivered. Consequently, activation of RhoA (a cytokinesis orchestrator) in the cortex is impaired, leading to the genesis of binuclear progeny (Figure 3A). In rat liver, the number of binuclear hepatocytes increases after weaning, suggesting an important connection between liver physiological characteristics and cytokinesis regulation.[77] Rats with low levels of circulating insulin exhibit reduced formation of binuclear polyploid hepatocytes, whereas rats injected with insulin exhibit an increase.[58,78] Suckling-to-weaning transition controls the initiation of cytokinesis failure mediated by the increase of insulin levels at this transiton.[58] In mouse liver, polyploidization already initiates around weaning,[53] making suckling/weaning transition less of a control factor than in the rat model. In fact, mice are less dependent on maternal feeding, with an unscheduled commencement of feeding (S. Celton-Morizur and C.D., unpublished data). How does insulin control polyploid development? The phosphatidylinositol 3-kinase/Akt pathway lies downstream of the insulin signal to regulate the ploidization process. Targeting Akt activity is sufficient to decrease the number of cytokinesis failure events.[58,78] Interestingly, a role for the phosphatidylinositol 3-kinase/Akt pathway has been described during the pathological polyploidization process. Akt1 regulates ploidy levels in vascular smooth muscle cells during hypertension.[79] Recent data demonstrate that E2F transcription factors are crucial for liver polyploidization during postnatal development. The E2F family contains both activator and repressor factors that affect the cell cycle.[80] Liver polyploidy is controlled antagonistically by E2F8 (repressor) and E2F1 (activator) factors.[81,82] A deficiency in *E2f8* prevents liver polyploidization by promoting cytokinesis; in contrast, a deficiency in *E2f1* enhances this process by inhibiting cytokinesis. E2F8 and E2F1 regulate antagonistically a transcriptional program in which cytokinetic genes controlling actin and microtubule networks are regulated.[81] E2F transcription factors have already been identified as key factors for the polyploidy process in *Arabidopsis* and *Drosophila* species.[83] Remarkably, E2F factors also drive an atypical cell cycle in mammalian cells, such as in placenta tissues. In this case, trophoblast giant cell polyploidization is dependent on two repressors, E2F7 and E2F8, and one activator, E2F1.[82]

Interestingly, experiments on stem cells and therapeutic applications have discovered that liver polyploidization can also take place through heterotypic cell fusion. In fact, *in vivo* fusion has been described as the main mechanism producing bone marrow–derived hepatocytes (BMDHs).[84–86] This process is promoted when there is liver damage and the appearance of BMDHs has even been associated with the

amelioration of hepatic dysfunction.[87] The generation of BMDHs is mainly the result of the fusion of a myeloid hematopoietic cell lineage with hepatocytes.[88] The existence of polyploid hepatocytes after homotypic cell fusion is still controversial. Willenbring and coworkers[87] looked for hepatocyte-hepatocyte fusion in the *Fah*-null mouse model of liver repopulation. In this model, hepatocyte-hepatocyte fusion was present in $<1$ to $3 \times 10^7$ cells. By contrast, Faggioli et al[89,90] show that homotypic fusion is one of the mechanisms contributing to hepatocyte polyploidy during postnatal liver growth in a chimeric mouse model. In principle, cytokinesis failure and cell fusion are not mutually exclusive and, thus, can both participate in liver polyploidization.

Finally, alteration of liver polyploidy has been observed in different genetic mouse models by silencing/overexpressing regulators of the cell cycle (*Ccne1/Ccne2*, *CDK1*, *p21*, and *Skp2*),[91–94] tumor-suppressor genes (*p53* and *pRB*),[95,96] DNA repair gene (*ERC1*),[97] and proto-oncogene (*c-myc*).[98,99] In these models, alterations of liver polyploidy take place; however, the mechanisms controlling these alterations are still unknown.

## Benefits and Costs of Being Polyploid

It is clearly demonstrated that hepatic tissue modifies its ploidy profile during physiological and pathological events. Do polyploid hepatocytes represent only a manifestation of liver growth and/or liver injury or do these cells have a specific function? What advantage does it give tissue to have a polyploid, rather than a diploid, contingent? The first thing to consider is plausible economy on energy resources by escaping mitosis.[56] This could be especially beneficial in rapid-growing tissues (eg, in liver regeneration after partial hepatectomy). Interestingly, in rat, the suckling-weaning period, linked to high-energy consumption, is connected with changes in hepatocyte proliferation and polyploidization.[58] Another theory often discussed is that liver polyploidization is associated with terminal differentiation and ageing.[61,100,101] In rodents, the genesis of polyploid hepatocytes after PH is linked to senescence-type changes, such as p21 and lipofuscin accumulation.[66] Moreover, the replicative activity of diploid and polyploid seems to differ *in vitro*. On stimulation with hepatocyte growth factor, the primary culture of diploid hepatocytes shows greater capacity for DNA synthesis compared with the polyploid contingent.[102] In contrast, several groups have demonstrated that polyploid hepatocytes are not engaged in senescence. For instance, tetraploid hepatocytes are highly regenerative after partial hepatectomy.[54] Moreover, *E2f8*$^{-/-}$ mice that have livers composed predominately of diploid hepatocytes do not reveal any significant differences in regenerative capacity compared with wild-type livers.[82] In mouse models used for repopulation (*Fah*$^{-/-}$ and urokinase plasminogen activator/severe combined immunodeficiency models), after transplantation, the proliferative capacity of the polyploid contingent is identical to the diploid contingent.[53,103,104] All these data suggest that liver polyploidization, in contrast to that in other tissues, is not linked to terminal differentiation. However, it would be interesting to establish if a polyploid hepatocyte with DNA and centrosome amplification performs DNA synthesis and completes cell division as fast as a diploid contingent.

Several examples from the literature illustrate that a polyploid state confers specific biological functions, as recently shown for *Drosophila*. Polyploidy in subperineurial glia is critical for the maintenance of the blood-brain barrier during larval brain development.[105] In mammals, megakaryocyte polyploidization is associated with up- and down-regulation of multiple genes, most being genes involved in terminal differentiation and platelet biogenesis.[106] In the liver, various studies have tried to establish whether polyploidization governs specific hepatocyte functions. It is generally known that hepatocytes specialize in a function based on their position along the portocentral axis of the liver lobule. This zonation of function mainly affects ammonia detoxification, glucose/energy metabolism, and xenobiotic metabolism.[107] Results from several laboratories have suggested the existence of polyploid zonation; periportal hepatocytes exhibit a lower polyploidy contingent than perivenous hepatocytes.[101,108,109] However, discrepant results have been reported, suggesting that similar proportions of polyploid hepatocytes are present in both areas.[82,110] The impact of polyploidization on hepatocyte functions has been characterized by comparing gene expression profiles of diploid and polyploid contingents (4n and 8n).[111] Only 50 candidate genes from a wide range of different biological processes were differently expressed. These data fit well with results obtained in budding yeast, in which only 17 genes showed significant ploidy-dependent increases or decreases in gene expression.[112] In the liver, polyploidy, in a physiological context, seems not to confer specific functions to hepatocytes. However, no studies have clearly defined if polyploid hepatocytes are more transcriptionally and/or post-transcriptionally active. Interestingly, Vinogradov and coworkers have performed comparative genome-scale analysis between liver tissues that present different levels of polyploidization. They observed a link between polyploidy and gene-controlling cell survival, DNA damage, hypoxia, and oxidative stress.[113,114] Finally, as already described, polyploid hepatocytes in mice and in humans generate aneuploid progenies (near-diploid, tetraploid, and octoploid cells) by a ploidy reversal process.[53,115] These cells carry genetic diversity that may operate as an adaptive mechanism to become more resistant to xenobiotic or nutritional injury. Remarkably, Duncan et al[116] recently showed, in a genetic mouse model, that aneuploidy is a mechanism of adaptation for hepatic injury linked to a metabolic disorder.

Finally, there is a long-standing hypothesis that genome multiplication is an adaptive response forming a buffer against oxidative stress and genotoxic damage.[31] This might

be especially important in tissue, such as the liver, that has a primary function of metabolizing and eliminating toxic compounds. To support this hypothesis, studies performed in the 1980s demonstrated that, in rats treated with DNA damage—inducing chemical carcinogens (diethylnitrosamine and 2-acetyl-aminofluorene), an expansion of only the diploid cell population prevails at the different stages of hepatocyte transformation,[76] suggesting that a polyploid contingent buffers from genotoxic damage. Polyploidization might also provide protection in cases of tumor-suppressor gene loss (eg, *p53* or *Rb*). Interestingly, loss of Rb and p53 during mouse liver development increased polyploidy content[95,96]; deletion of these two genes is not sufficient for spontaneous tumorigenesis.[117]

## Conclusion

The liver is a fascinating organ that, compared with other tissues, can deal with a high proliferative capacity and the presence of polyploid and aneuploid hepatocytes. Further investigations will increase our understanding of the functional consequences of hepatocyte polyploidy during development and aging and should also offer insights into hepatic pathological conditions, such as hepatocarcinoma.

## References


1. Ramsey J, Schemske DW: Pathways, mechanisms and rates of polyploid formation in flowering plants. Annu Rev Ecol Syst 1998, 29:467—501
2. Leitch AR, Leitch IJ: Genomic plasticity and the diversity of polyploid plants. Science 2008, 320:481—483
3. Otto SP: The evolutionary consequences of polyploidy. Cell 2007, 131:452—462
4. Guc-Scekic M, Milasin J, Stevanovic M, Stojanov LJ, Djordjevic M: Tetraploidy in a 26-month-old girl (cytogenetic and molecular studies). Clin Genet 2002, 61:62—65
5. Jacobs PA, Szulman AE, Funkhouser J, Matsuura JS, Wilson CC: Human triploidy: relationship between parental origin of the additional haploid complement and development of partial hydatidiform mole. Ann Hum Genet 1982, 46:223—231
6. Gallardo MH, Bickham JW, Honeycutt RL, Ojeda RA, Kohler N: Discovery of tetraploidy in a mammal. Nature 1999, 401:341
7. Davoli T, de Lange T: The causes and consequences of polyploidy in normal development and cancer. Annu Rev Cell Dev Biol 2011, 27: 585—610
8. van der Heijden FL, James J: Polyploidy in the human myometrium. Z Mikrosk Anat Forsch 1975, 89:18—26
9. Hixon ML, Gualberto A: Vascular smooth muscle polyploidization: from mitotic checkpoints to hypertension. Cell Cycle 2003, 2: 105—110
10. Vliegen HW, Eulderink F, Bruschke AV, van der Laarse A, Cornelisse CJ: Polyploidy of myocyte nuclei in pressure overloaded human hearts: a flow cytometric study in left and right ventricular myocardium. Am J Cardiovasc Pathol 1995, 5:27—31
11. Auer GU, Backdahl M, Forsslund GM, Askensten UG: Ploidy levels in nonneoplastic and neoplastic thyroid cells. Anal Quant Cytol Histol 1985, 7:97—106
12. Ganem NJ, Storchova Z, Pellman D: Tetraploidy, aneuploidy and cancer. Curr Opin Genet Dev 2007, 17:157—162
13. Taylor MV: Muscle differentiation: how two cells become one. Curr Biol 2002, 12:R224—R228
14. Vignery A: Macrophage fusion: molecular mechanisms. Methods Mol Biol 2008, 475:149—161
15. Larsson LI, Bjerregaard B, Talts JF: Cell fusions in mammals. Histochem Cell Biol 2008, 129:551—561
16. Rochlin K, Yu S, Roy S, Baylies MK: Myoblast fusion: when it takes more to make one. Dev Biol 2010, 341:66—83
17. Duelli D, Lazebnik Y: Cell-to-cell fusion as a link between viruses and cancer. Nat Rev Cancer 2007, 7:968—976
18. Gao P, Zheng J: High-risk HPV E5-induced cell fusion: a critical initiating event in the early stage of HPV-associated cervical cancer. Virol J 2010, 7:238
19. Edgar BA, Orr-Weaver TL: Endoreplication cell cycles: more for less. Cell 2001, 105:297—306
20. Duronio RJ, O'Farrell PH: Developmental control of the G1 to S transition in Drosophila: cyclin E is a limiting downstream target of E2F. Genes Dev 1995, 9:1456—1468
21. Narbonne-Reveau K, Senger S, Pal M, Herr A, Richardson HE, Asano M, Deak P, Lilly MA: APC/CFzr/Cdh1 promotes cell cycle progression during the Drosophila endocycle. Development 2008, 135:1451—1461
22. Parisi T, Beck AR, Rougier N, McNeil T, Lucian L, Werb Z, Amati B: Cyclins E1 and E2 are required for endoreplication in placental trophoblast giant cells. EMBO J 2003, 22:4794—4803
23. Ullah Z, Kohn MJ, Yagi R, Vassilev LT, DePamphilis ML: Differentiation of trophoblast stem cells into giant cells is triggered by p57/Kip2 inhibition of CDK1 activity. Genes Dev 2008, 22: 3024—3036
24. Fox DT, Duronio RJ: Endoreplication and polyploidy: insights into development and disease. Development 2013, 140:3—12
25. Davoli T, de Lange T: Telomere-driven tetraploidization occurs in human cells undergoing crisis and promotes transformation of mouse cells. Cancer Cell 2012, 21:765—776
26. Brito DA, Rieder CL: Mitotic checkpoint slippage in humans occurs via cyclin B destruction in the presence of an active checkpoint. Curr Biol 2006, 16:1194—1200
27. Rieder CL: Mitosis in vertebrates: the G2/M and M/A transitions and their associated checkpoints. Chromosome Res 2011, 19:291—306
28. Caldwell CM, Kaplan KB: The role of APC in mitosis and in chromosome instability. Adv Exp Med Biol 2009, 656:51—64
29. Dikovskaya D, Schiffmann D, Newton IP, Oakley A, Kroboth K, Sansom O, Jamieson TJ, Meniel V, Clarke A, Nathke IS: Loss of APC induces polyploidy as a result of a combination of defects in mitosis and apoptosis. J Cell Biol 2007, 176:183—195
30. Fededa JP, Gerlich DW: Molecular control of animal cell cytokinesis. Nat Cell Biol 2012, 14:440—447
31. Storchova Z, Pellman D: From polyploidy to aneuploidy, genome instability and cancer. Nat Rev Mol Cell Biol 2004, 5:45—54
32. Eggert US, Mitchison TJ, Field CM: Animal cytokinesis: from parts list to mechanisms. Annu Rev Biochem 2006, 75:543—566
33. Aylon Y, Oren M: p53: Guardian of ploidy. Mol Oncol 2012, 5:315—323
34. Cimini D, Moree B, Canman JC, Salmon ED: Merotelic kinetochore orientation occurs frequently during early mitosis in mammalian tissue cells and error correction is achieved by two different mechanisms. J Cell Sci 2003, 116:4213—4225
35. Hayashi MT, Karlseder J: DNA damage associated with mitosis and cytokinesis failure. Oncogene 2013, 32:4593—4601
36. Steigemann P, Wurzenberger C, Schmitz MH, Held M, Guizetti J, Maar S, Gerlich DW: Aurora B-mediated abscission checkpoint protects against tetraploidization. Cell 2009, 136:473—484
37. Lacroix B, Maddox AS: Cytokinesis, ploidy and aneuploidy. J Pathol 2012, 226:338—351
38. Vinciguerra P, Godinho SA, Parmar K, Pellman D, D'Andrea AD: Cytokinesis failure occurs in Fanconi anemia pathway-deficient murine and human bone marrow hematopoietic cells. J Clin Invest 2010, 120:3834—3842

39. Li F, Wang X, Capasso JM, Gerdes AM: Rapid transition of cardiac myocytes from hyperplasia to hypertrophy during postnatal development. J Mol Cell Cardiol 1996, 28:1737−1746
40. Clubb FJ Jr., Bishop SP: Formation of binucleated myocardial cells in the neonatal rat: an index for growth hypertrophy. Lab Invest 1984, 50:571−577
41. Liu Z, Yue S, Chen X, Kubin T, Braun T: Regulation of cardiomyocyte polyploidy and multinucleation by CyclinG1. Circ Res 2010, 106:1498−1506
42. Engel FB, Schebesta M, Keating MT: Anillin localization defect in cardiomyocyte binucleation. J Mol Cell Cardiol 2006, 41:601−612
43. Ahuja P, Sdek P, MacLellan WR: Cardiac myocyte cell cycle control in development, disease, and regeneration. Physiol Rev 2007, 87: 521−544
44. Nguyen HG, Ravid K: Polyploidy: mechanisms and cancer promotion in hematopoietic and other cells. Adv Exp Med Biol 2010, 676: 105−122
45. Ravid K, Lu J, Zimmet JM, Jones MR: Roads to polyploidy: the megakaryocyte example. J Cell Physiol 2002, 190:7−20
46. Gao Y, Smith E, Ker E, Campbell P, Cheng EC, Zou S, Lin S, Wang L, Halene S, Krause DS: Role of RhoA-specific guanine exchange factors in regulation of endomitosis in megakaryocytes. Dev Cell 2012, 22:573−584
47. Eliades A, Papadantonakis N, Ravid K: New roles for cyclin E in megakaryocytic polyploidization. J Biol Chem 2010, 285: 18909−18917
48. Si-Tayeb K, Lemaigre FP, Duncan SA: Organogenesis and development of the liver. Dev Cell 2010, 18:175−189
49. Jungermann K, Kietzmann T: Zonation of parenchymal and nonparenchymal metabolism in liver. Annu Rev Nutr 1996, 16:179−203
50. Epstein CJ: Cell size, nuclear content and the development of polyploidy in the mammalian liver. Proc Natl Acad Sci U S A 1967, 57: 327−334
51. Gerlyng P, Abyholm A, Grotmol T, Erikstein B, Huitfeldt HS, Stokke T, Seglen PO: Binucleation and polyploidization patterns in developmental and regenerative rat liver growth. Cell Prolif 1993, 26: 557−565
52. Guidotti JE, Bregerie O, Robert A, Debey P, Brechot C, Desdouets C: Liver cell polyploidization: a pivotal role for binuclear hepatocytes. J Biol Chem 2003, 278:19095−19101
53. Duncan AW, Taylor MH, Hickey RD, Hanlon Newell AE, Lenzi ML, Olson SB, Finegold MJ, Grompe M: The ploidy conveyor of mature hepatocytes as a source of genetic variation. Nature 2010, 467:707−710
54. 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, 22:1166−1175
55. Duncan AW: Aneuploidy, polyploidy and ploidy reversal in the liver. Semin Cell Dev Biol 2013, 24:347−356
56. Anatskaya OV, Vinogradov AE, Kudryavtsev BN: Hepatocyte polyploidy and metabolism/life-history traits: hypotheses testing. J Theor Biol 1994, 168:191−199
57. Barbason H, Herens C, Mormont MC, Bouzahzah B: Circadian synchronization of hepatocyte proliferation in young rats: the role played by adrenal hormones. Cell Tissue Kinet 1987, 20:57−67
58. 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, 119:1880−1887
59. Dallman PR, Spirito RA, Siimes MA: Diurnal patterns of DNA synthesis in the rat: modification by diet and feeding schedule. J Nutr 1974, 104:1234−1241
60. 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, 9:939−945
61. Kudryavtsev BN, Kudryavtseva MV, Sakuta GA, Stein GI: Human hepatocyte polyploidization kinetics in the course of life cycle. Virchows Arch B Cell Pathol Incl Mol Pathol 1993, 64: 387−393
62. Toyoda H, Bregerie O, Vallet A, Nalpas B, Pivert G, Brechot C, Desdouets C: Changes to hepatocyte ploidy and binuclearity profiles during human chronic viral hepatitis. Gut 2005, 54:297−302
63. Deschenes J, Valet JP, Marceau N: The relationship between cell volume, ploidy, and functional activity in differentiating hepatocytes. Cell Biophys 1981, 3:321−334
64. Martin NC, McCullough CT, Bush PG, Sharp L, Hall AC, Harrison DJ: Functional analysis of mouse hepatocytes differing in DNA content: volume, receptor expression, and effect of IFNgamma. J Cell Physiol 2002, 191:138−144
65. Faktor VM, Uryvaeva IV: Mitotic cycle of diploid and polyploid cells of regenerating rat liver [in Russian]. Tsitologiia 1972, 14:868−872
66. Faktor VM, Uryvaeva IV: Progressive polyploidy in mouse liver following repeated hepatectomy [in Russian]. Tsitologiia 1975, 17: 909−916
67. Sigal SH, Rajvanshi P, Gorla GR, Sokhi RP, Saxena R, Gebhard DR Jr., Reid LM, Gupta S: Partial hepatectomy-induced polyploidy attenuates hepatocyte replication and activates cell aging events. Am J Physiol 1999, 276:G1260−G1272
68. Madra S, Styles J, Smith AG: Perturbation of hepatocyte nuclear populations induced by iron and polychlorinated biphenyls in C57BL/10ScSn mice during carcinogenesis. Carcinogenesis 1995, 16:719−727
69. Muramatsu Y, Yamada T, Moralejo DH, Mochizuki H, Sogawa K, Matsumoto K: Increased polyploid incidence is associated with abnormal copper accumulation in the liver of LEC mutant rat. Res Commun Mol Pathol Pharmacol 2000, 107:129−136
70. Yamada T, Sogawa K, Kim JK, Izumi K, Suzuki Y, Muramatsu Y, Sumida T, Hamakawa H, Matsumoto K: Increased polyploidy, delayed mitosis and reduced protein phosphatase-1 activity associated with excess copper in the Long Evans Cinnamon rat. Res Commun Mol Pathol Pharmacol 1998, 99:283−304
71. Denchi EL, Celli G, de Lange T: Hepatocytes with extensive telomere deprotection and fusion remain viable and regenerate liver mass through endoreduplication. Genes Dev 2006, 20:2648−2653
72. Toyoda H, Kumada T, Bregerie O, Brechot C, Desdouets C: Conserved balance of hepatocyte nuclear DNA content in mononuclear and binuclear hepatocyte populations during the course of chronic viral hepatitis. World J Gastroenterol 2006, 12:4546−4548
73. Gorla GR, Malhi H, Gupta S: Polyploidy associated with oxidative injury attenuates proliferative potential of cells. J Cell Sci 2001, 114: 2943−2951
74. Nakatani T, Inouye M, Mirochnitchenko O: Overexpression of antioxidant enzymes in transgenic mice decreases cellular ploidy during liver regeneration. Exp Cell Res 1997, 236:137−146
75. Diez-Fernandez C, Sanz N, Alvarez AM, Zaragoza A, Cascales M: Influence of aminoguanidine on parameters of liver injury and regeneration induced in rats by a necrogenic dose of thioacetamide. Br J Pharmacol 1998, 125:102−108
76. Celton-Morizur S, Desdouets C: Polyploidization of liver cells. Adv Exp Med Biol 2010, 676:123−135
77. Margall-Ducos G, Celton-Morizur S, Couton D, Bregerie O, Desdouets C: Liver tetraploidization is controlled by a new process of incomplete cytokinesis. J Cell Sci 2007, 120:3633−3639
78. Celton-Morizur S, Merlen G, Couton D, Desdouets C: Polyploidy and liver proliferation: central role of insulin signaling. Cell Cycle 2010, 9:460−466
79. Hixon ML, Muro-Cacho C, Wagner MW, Obejero-Paz C, Millie E, Fujio Y, Kureishi Y, Hassold T, Walsh K, Gualberto A: Akt1/PKB upregulation leads to vascular smooth muscle cell hypertrophy and polyploidization. J Clin Invest 2000, 106:1011−1020
80. Wong JV, Dong P, Nevins JR, Mathey-Prevot B, You L: Network calisthenics: control of E2F dynamics in cell cycle entry. Cell Cycle 2011, 10:3086−3094
81. Chen HZ, Ouseph MM, Li J, Pecot T, Chokshi V, Kent L, Bae S, Byrne M, Duran C, Comstock G, Trikha P, Mair M, Senapati S,

Martin CK, Gandhi S, Wilson N, Liu B, Huang YW, Thompson JC, Raman S, Singh S, Leone M, Machiraju R, Huang K, Mo X, Fernandez S, Kalaszczynska I, Wolgemuth DJ, Sicinski P, Huang T, Jin V, Leone G: Canonical and atypical E2Fs regulate the mammalian endocycle. Nat Cell Biol 2012, 14:1192–1202

82. Pandit SK, Westendorp B, Nantasanti S, van Liere E, Tooten PC, Cornelissen PW, Toussaint MJ, Lamers WH, de Bruin A: E2F8 is essential for polyploidization in mammalian cells. Nat Cell Biol 2012, 14:1181–1191
83. Meserve JH, Duronio RJ: Atypical E2Fs drive atypical cell cycles. Nat Cell Biol 2012, 14:1124–1125
84. Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K, Lois C, Morrison SJ, Alvarez-Buylla A: Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature 2003, 425:968–973
85. Vassilopoulos G, Wang PR, Russell DW: Transplanted bone marrow regenerates liver by cell fusion. Nature 2003, 422:901–904
86. Wang X, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al-Dhalimy M, Lagasse E, Finegold M, Olson S, Grompe M: Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature 2003, 422:897–901
87. Willenbring H, Bailey AS, Foster M, Akkari Y, Dorrell C, Olson S, Finegold M, Fleming WH, Grompe M: Myelomonocytic cells are sufficient for therapeutic cell fusion in liver. Nat Med 2004, 10:744–748
88. Camargo FD, Finegold M, Goodell MA: Hematopoietic myelomonocytic cells are the major source of hepatocyte fusion partners. J Clin Invest 2004, 113:1266–1270
89. Faggioli F, Sacco MG, Susani L, Montagna C, Vezzoni P: Cell fusion is a physiological process in mouse liver. Hepatology 2008, 48: 1655–1664
90. Faggioli F, Vezzoni P, Montagna C: Single-cell analysis of ploidy and centrosomes underscores the peculiarity of normal hepatocytes. PLoS One 2011, 6:e26080
91. Diril MK, Ratnacaram CK, Padmakumar VC, Du T, Wasser M, Coppola V, Tessarollo L, Kaldis P: 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, 109:3826–3831
92. Minamishima YA, Nakayama K: Recovery of liver mass without proliferation of hepatocytes after partial hepatectomy in Skp2-deficient mice. Cancer Res 2002, 62:995–999
93. Nevzorova YA, Tschaharganeh D, Gassler N, Geng Y, Weiskirchen R, Sicinski P, Trautwein C, Liedtke C: Aberrant cell cycle progression and endoreplication in regenerating livers of mice that lack a single E-type cyclin. Gastroenterology 2009, 137: 691–703, 703.e1–6
94. Wu H, Wade M, Krall L, Grisham J, Xiong J, Van Dyke T: Targeted in vivo expression of the cyclin dependent kinase inhibitor p21 halts hepatocyte cell cycle progression, postnatal liver development and regeneration. Genes Dev 1996, 10:245–260
95. Kurinna S, Stratton SA, Coban Z, Schumacher JM, Grompe M, Duncan AW, Barton MC: p53 Regulates a mitotic transcription program and determines ploidy in normal mouse liver. Hepatology 2013, 57:2004–2013
96. Mayhew CN, Bosco EE, Fox SR, Okaya T, Tarapore P, Schwemberger SJ, Babcock GF, Lentsch AB, Fukasawa K, Knudsen ES: Liver-specific pRB loss results in ectopic cell cycle entry and aberrant ploidy. Cancer Res 2005, 65:4568–4577
97. Nunez F, Chipchase MD, Clarke AR, Melton DW: Nucleotide excision repair gene (ERCC1) deficiency causes G(2) arrest in hepatocytes and a reduction in liver binucleation: the role of p53 and p21. FASEB J 2000, 14:1073–1082
98. Baena E, Gandarillas A, Vallespinos M, Zanet J, Bachs O, Redondo C, Fabregat I, Martinez AC, de Alboran IM: c-Myc regulates cell size and ploidy but is not essential for postnatal proliferation in liver. Proc Natl Acad Sci U S A 2005, 102:7286–7291
99. Conner EA, Lemmer ER, Sanchez A, Factor VM, Thorgeirsson SS: E2F1 blocks and c-Myc accelerates hepatic ploidy in transgenic mouse models. Biochem Biophys Res Commun 2003, 302:114–120
100. Gupta S: Hepatic polyploidy and liver growth control. Semin Cancer Biol 2000, 10:161–171
101. Schmucker DL: Hepatocyte fine structure during maturation and senescence. J Electron Microsc Tech 1990, 14:106–125
102. Rajvanshi P, Liu D, Ott M, Gagandeep S, Schilsky ML, Gupta S: Fractionation of rat hepatocyte subpopulations with varying metabolic potential, proliferative capacity, and retroviral gene transfer efficiency. Exp Cell Res 1998, 244:405–419
103. 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, 155:2135–2143
104. Weglarz TC, Sandgren EP: Timing of hepatocyte entry into DNA synthesis after partial hepatectomy is cell autonomous. Proc Natl Acad Sci U S A 2000, 97:12595–12600
105. Unhavaithaya Y, Orr-Weaver TL: Polyploidization of glia in neural development links tissue growth to blood-brain barrier integrity. Genes Dev 2012, 26:31–36
106. Raslova H, Kauffmann A, Sekkai D, Ripoche H, Larbret F, Robert T, Le Roux DT, Kroemer G, Debili N, Dessen P, Lazar V, Vainchenker W: Interrelation between polyploidization and megakaryocyte differentiation: a gene profiling approach. Blood 2007, 109:3225–3234
107. Benhamouche S, Decaens T, Godard C, Chambrey R, Rickman DS, Moinard C, Vasseur-Cognet M, Kuo CJ, Kahn A, Perret C, Colnot S: Apc tumor suppressor gene is the "zonation-keeper" of mouse liver. Dev Cell 2006, 10:759–770
108. Asahina K, Shiokawa M, Ueki T, Yamasaki C, Aratani A, Tateno C, Yoshizato K: Multiplicative mononuclear small hepatocytes in adult rat liver: their isolation as a homogeneous population and localization to periportal zone. Biochem Biophys Res Commun 2006, 342: 1160–1167
109. Gandillet A, Alexandre E, Holl V, Royer C, Bischoff P, Cinqualbre J, Wolf P, Jaeck D, Richert L: Hepatocyte ploidy in normal young rat. Comp Biochem Physiol A Mol Integr Physiol 2003, 134:665–673
110. Margall-Ducos G, Morizur-Celton S, Couton D, Bregerie O, Desdouets C: Liver tetraploidization is controlled by a new process of incomplete cytokinesis. J Cell Sci 2007, 120:3633–3639
111. 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, 18:617–626
112. Galitski T, Saldanha AJ, Styles CA, Lander ES, Fink GR: Ploidy regulation of gene expression. Science 1999, 285:251–254
113. Anatskaya OV, Vinogradov AE: Genome multiplication as adaptation to tissue survival: evidence from gene expression in mammalian heart and liver. Genomics 2007, 89:70–80
114. Anatskaya OV, Vinogradov AE: Somatic polyploidy promotes cell function under stress and energy depletion: evidence from tissue-specific mammal transcriptome. Funct Integr Genomics 2010, 10:433–446
115. Duncan AW, Hanlon Newell AE, Smith L, Wilson EM, Olson SB, Thayer MJ, Strom SC, Grompe M: Frequent aneuploidy among normal human hepatocytes. Gastroenterology 2012, 142:25–28
116. Duncan AW, Hanlon Newell AE, Bi W, Finegold MJ, Olson SB, Beaudet AL, Grompe M: Aneuploidy as a mechanism for stress-induced liver adaptation. J Clin Invest 2012, 122:3307–3315
117. McClendon AK, Dean JL, Ertel A, Fu Z, Rivadeneira DB, Reed CA, Bourgo RJ, Witkiewicz A, Addya S, Mayhew CN, Grimes HL, Fortina P, Knudsen ES: RB and p53 cooperate to prevent liver tumorigenesis in response to tissue damage. Gastroenterology 2011, 141:1439–1450