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REVIEW

Polyploidization in Liver Tissue

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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 animals³ (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

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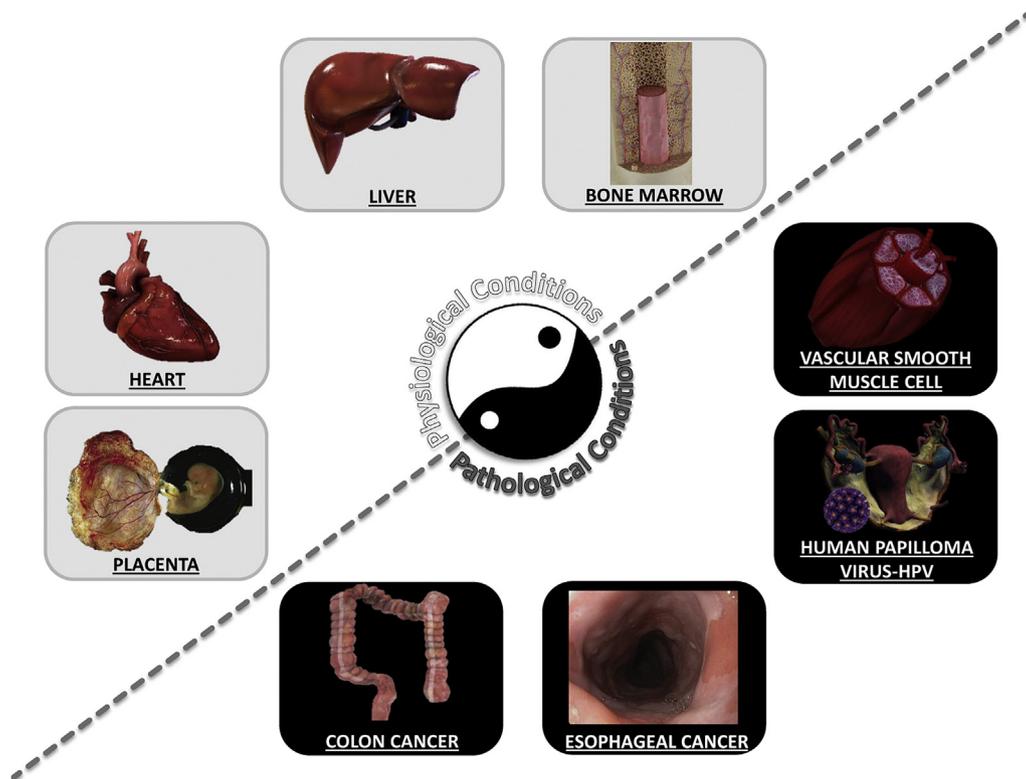


Figure 1 Polyploidy during physiological and pathological processes. Polyploid cells are generated during physiological processes, such as embryogenesis (placenta-trophoblast giant cells), postnatal development (heart-cardiomyocytes or liver-hepatocytes), and terminal differentiation (bone marrow—megakaryocytes); 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).

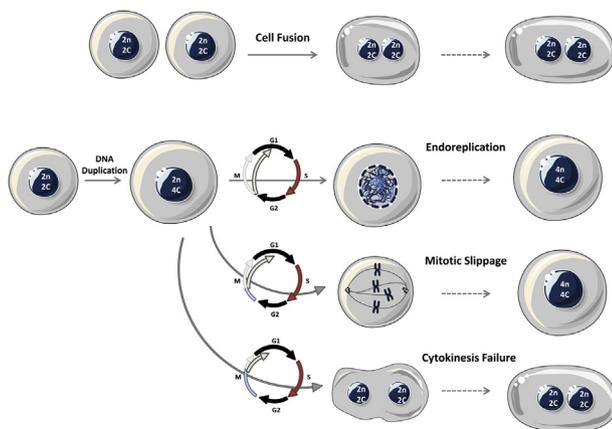


Figure 2 Mechanisms leading to the genesis of tetraploid cells. Tetraploid cells can be generated by cell-to-cell fusion, or by abortive cell cycles after DNA replication: endoreplication, mitotic slippage, or cytokinesis failure. Although cell fusion and cytokinesis failure produce binuclear progeny, 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 G₂/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 G₁ state, from which they die, arrest in G₁, 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 Kaplan²⁸ 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 Maddox³⁷ 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 al³⁸ 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 G₁ has been identified as an important component of the molecular machinery controlling this process. Directed expression of cyclin G₁ in neonatal cardiomyocytes promotes G₁/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 cells⁴⁸; 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, $2 \times 2n$ or $2 \times 4n$). 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

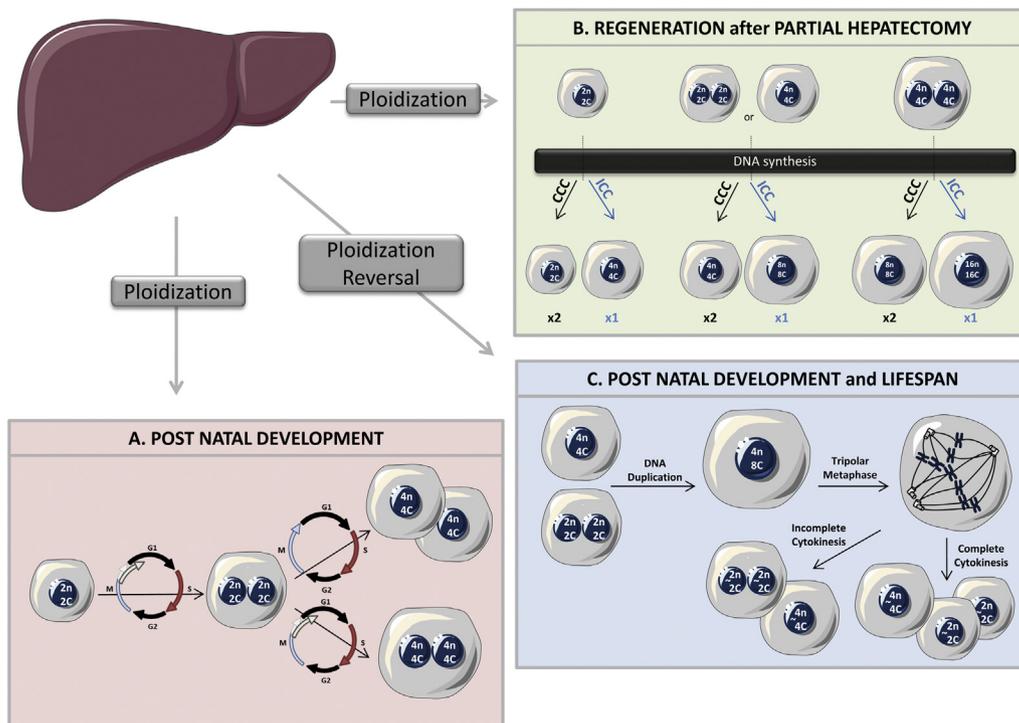


Figure 3 Liver parenchyma and hepatocyte ploidy. During postnatal development, polyploidization takes place. Diploid hepatocytes can mostly go through a cell cycle with karyokinesis, but failure of cytokinesis results in a binuclear tetraploid hepatocyte that reenters the cell cycle and either undergoes 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 $2 \times 2n$ 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 through 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 colleagues⁷³ 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 transition.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 coworkers⁸⁷ 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 3×10^7 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 predominantly 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 al¹¹⁶ 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.

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