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Abstract | Polyploidy (or whole-genome duplication) is the condition of having more than two basic sets of chromosomes. Polyploidization is well tolerated in many species and can lead to specific biological functions. In mammals, programmed polyploidization takes place during development in certain tissues such as the heart and placenta and is considered to be a feature of differentiation. However, unscheduled polyploidization can cause genomic instability and has been observed in pathological conditions, such as cancer. Polyploidy of the liver parenchyma was first described more than 100 years ago. It's one of the few mammalian organs that display changes in polyploidy during homeostasis, regeneration and in response to damage. In the human liver, around 30% of hepatocytes are polyploid. The polyploidy of hepatocytes results from both nuclear polyploidy (an increase in the amount of DNA per nucleus) and cellular polyploidy (an increase in the number of nuclei per cell). In this Review, we discuss the regulation of polyploidy in liver development and pathophysiology. We also provide an overview of current knowledge about the mechanisms of hepatocyte polyploidization, its biological importance and the fate of polyploid hepatocytes during liver tumorigenesis.

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Key points

- Polyploidy, a condition in which cells contain more than two sets of homologous chromosomes, is a well-known feature of mammalian hepatocytes.
- Polyploidy is defined on the basis of the DNA content of each nucleus (nuclear ploidy; for example, 2n, 4n or 8n) and the number of nuclei per cell (cellular ploidy).
- The adult liver contains a heterogeneous mixture of diploid and polyploid hepatocytes.
- The liver is one of the few mammalian organs that display changes in ploidy during normal homeostasis, regeneration and in response to damage.
- Polyploid state could provide protection from tumorigenesis by providing extra copies of tumour suppressor copies.
- Amplification of nuclear ploidy within liver tumours is associated with a poor prognosis.

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Introduction

The chromosome houses the genetic information specific to all living beings. Each species has a specific number, *n*, of chromosomes in its haploid genome. Thus, *Homo sapiens* cells have $n = 23$ chromosomes and *Mus musculus* cells have $n = 20$ chromosomes in their haploid genome [1]. Eukaryotic organisms typically have two complete sets of homologous chromosomes. However, the number of a set of chromosomes can differ between cells or species. Cells with only one copy of each chromosome (*n*) are described as haploid, those with two copies are diploid (2*n*), three copies are triploid (3*n*), four copies are tetraploid (4*n*), and so on (FIG. 1A). The presence of more than two complete sets of chromosomes is known as polyploidy or whole-genome duplication [2]. The polyploid state should not be confused with aneuploidy, in which the copy number of whole chromosomes or chromosome segments is modified, either by a gain or a loss [3]. In polyploid species, the additional chromosome sets can have different origins. Allopolyploidy arises through the fusion of two or more cells with distantly related genomes (that is, from different species). Autopolyploidy arises through the duplication of a single genome or the fusion of closely related genomes (that is, from the same species). Finally, the number of chromosome sets can be amplified within a single nucleus (mononucleate polyploid populations), defining the nuclear ploidy, or, as in some polyploid cells, the genetic material can be distributed between two or more nuclei, defining the cellular ploidy (FIG. 1B).

Polyploidy is not rare in eukaryotes, and is now considered a common mode of speciation, with consequences for evolution and biodiversity [4-7]. Indeed, polyploidy is a feature of plant genomes, contributing to variations in both genome size and gene content [8-10]. Polyploidy has been observed in most plant groups, but it is most frequent in angiosperms [11-13]. It has also been reported in some insects, fishes, amphibians and reptiles [7, 14]. In mammals, whole-

organism polyploidization is rare, as it typically leads to embryonic resorption or spontaneous abortion [15-17]. However, the red viscacha rat (*Tympanoctomys barrerae*) and its close relatives, which are fully tetraploid, are the exception to the rule [18]. The emergence of polyploid cells in mammals can be associated with the development and differentiation of certain tissues. For instance, polyploid cells are present in the heart (cardiomyocytes: 4*n*), placenta (trophoblast giant cells: 8*n* to 64*n*), bone marrow (megakaryocytes: 16*n* to 128*n*), pancreas (acinar cells: 4*n*) [19, 20] and liver (hepatocytes: 4*n* to 8*n*) [21-23]. Over the past decade, several breakthroughs have occurred in determining the role of polyploidy in regulating organ size, tissue regeneration and repair [24, 25]. In *Drosophila melanogaster* and some vertebrate tissues, polyploidy provides an alternative means of compensating for cell loss, particularly in post-mitotic tissues that lack stem cells [25]. For example, polyploidy has a crucial role in heart and kidney regeneration and repair processes in mammals [25-28]. One major concern is the association of an accumulation of polyploid contingents with many age-related diseases, including arterial hypertension, hyperthyroidism, metabolic disorders and cancer [29-33]. Proliferating polyploid cells have been shown to be genetically unstable, thereby potentially facilitating tumour development [21, 34]. In addition, there is growing evidence to suggest that polyploid intermediates play a key part in shaping the composition of cancer genomes: most solid tumours have polyploid or near-polyploid karyotypes [35, 36].

Thus, several key questions arise. Does polyploidy confer selective benefits and a protective role in evolution, or does it generate a key contingent of cells that underlie tumorigenesis? This Review focuses on the duality of the polyploid state, and on the mechanisms leading to it. In this Review, we will first introduce the different mechanisms leading to polyploidy. Next, we will examine how polyploid hepatocytes are generated during physiological and pathological situations. Finally, we will discuss the effects of polyploidy on liver function.

[H1] Mechanisms leading to polyploidy

How does a diploid cell become polyploid? In a physiological or pathological context, various mechanisms can promote the genesis of polyploid cells: endoreplication, cytokinesis failure and cell fusion, which is not dependent on the cell cycle and division (FIG. 2).

[H2] Cell fusion

Fusion can occur between cells of the same type (homotypic) or cells of different origins (heterotypic) (FIG. 2A). Cells that fuse without nuclear fusion are described as syncytia. The major cell fusion event that modifies ploidy is fertilization (an example of heterotypic cell fusion) [37]. Cell fusion is also a key process in tissue development and homeostasis. For example, myoblasts fuse to form myotubes in muscle, macrophages fuse to form osteoclasts in bone, and cytotrophoblasts fuse to form placental syncytiotrophoblasts [38-40]. Cell fusion mechanisms have been analysed in detail in several experimental models, in particular in myoblast differentiation [41]. Cell fusion requires cell–cell adhesion via the repulsive charges of the two cells' membranes, followed by destabilization of the opposing lipid bilayers, leading to fusion, pore formation and expansion. Myoblasts use actin-propelled membrane protrusions to promote fusogenic protein engagement and fusion pore formation [42]. Cell fusion also has a key role in wound healing and tissue regeneration. In injured tissues, bone marrow-derived cells can fuse to differentiated cells to form hybrids with regenerative potential [43]. Such heterotypic cell fusion has been observed in various human and mice organs, including the muscle, brain, retina, intestine and liver, and it has been shown to participate in the re-establishment of tissue function [44-48].

Viral infections can also have an important role in the formation of polyploid cells [49]. The best-documented example is that of human papillomavirus (HPV), the major aetiological agent of cervical cancer [50]. The expression of the viral HPV-16 E5 oncoprotein at the surface of infected cervical epithelial cells is sufficient for the formation of tetraploid binucleate cells

[51, 52]. Tetraploid cervical cells are observed in premalignant lesions of human cervical cancer and it has been established as a prognostic factor that allows estimating the relative progression risk into more advanced lesion [53]. Interestingly, women diagnosed as low-grade squamous intraepithelial lesion/HPV-positive exhibit elevated levels of tetraploid cervical cells compared with normal/HPV-negative women, indicating that formation of tetraploid cells is obviously associated with HPV infection [54].

[H2] Endoreplication

The canonical cell cycle of mammals has four successive phases: G1, S, G2 and M (FIG. 2B). This process is governed by the cyclin-dependent kinases (CDKs), a family of serine–threonine protein kinases. CDK activity is dependent on association with non-catalytic regulatory subunits called cyclins. CDK–cyclin complexes phosphorylate specific substrates to induce DNA replication (which is driven by the CDK responsible for S phase entry, S-CDK) and mitosis (which is driven by the CDK responsible for M phase entry, M-CDK), the two major events of the cell division cycle [55]. In mammals, the S and M phases are controlled by CDK2 associated with cyclins E and A, and by CDK1 associated with cyclins B and A, respectively. These two steps are coupled, such that mitosis phase cannot begin until S phase has been completed. Endoreplication is an alternative cell cycle in which cells successively replicate their genomes in the absence of cell division [56-59]. This process leads to the genesis of mononucleate polyploid progenies. There are two variants of the endoreplication process: endocycles and endomitosis (FIG. 2C). During endocycles, cells alternate between G and S phases, whereas, during endomitosis, cells abort mitosis by skipping metaphase, anaphase or cytokinesis [60]. Several elegant reviews have highlighted the mechanisms that regulate endocycles in different species and cell types [25, 57, 61]. The key event that triggers endocycles is the inhibition of entry into mitosis. This inhibition might be mediated by the proteolysis of mitotic cyclins under the control of an E3 ubiquitin ligase, the APC/C (anaphase-promoting

complex; also known as the cyclosome). M-CDK can also be inhibited by interactions with cyclin kinase inhibitors (CKIs). A second crucial event is the oscillation of S-CDK activity between low and high levels in the G and S phases, respectively, which is essential for genome reduplication in the absence of cell division. One of the best-characterized examples of an endocycle in mammals is observed in the trophoblast giant cells. Trophoblast giant cell polyploidization is crucial for implantation and for the modulation of post-implantation placentation [62]. In particular, the accumulation of the CKIs p57 and p21 is essential for endocycle induction, as it enhances cyclin B degradation and inhibits CDK1 activity [63, 64]. Also, E2F transcription factors are required for the regulation of trophoblast giant cell polyploidization [65, 66]. This point is discussed in more detail later.

The observation in different experimental models of a connection between genome instability and endoreplication cycles is a major source of concern [57, 67]. For instance, double-strand break induction is common in the leaves of *Arabidopsis thaliana* plants subjected to UV irradiation. Induction of the DNA damage response results in G2–M arrest. If the DNA damage response persists, endoreplication is triggered [68, 69]. In this context, it has been suggested that endoreplication is a strategy used by the plant to sustain growth under genotoxic stress. In human and mouse cell lines, that have high amounts of dysfunctional telomeres, endoreplication is induced by p53/pRb [70, 71]. This endoreplication requires persistent serine-protein kinase ATM and/or ATR (ATM and RAD3-related)-dependent signalling, which is induced by irreparably damaged telomeres. Prolonged cell cycle arrest in G2 leads to mitosis being bypassed, which results in the genesis of mononucleate tetraploid progenies [70, 71].

Endomitosis has been linked to problems in metaphase–anaphase transition. Thus, cancer cells treated with antimitotic drugs that target microtubule dynamics (for example, taxanes, vinca alkaloids and epothilones) are blocked in metaphase owing to spindle assembly

checkpoint (SAC) activation [72]. The SAC prevents passage through anaphase until all chromosomes are properly attached to kinetochores [73]. Sustained SAC activation results in either cell death through a phenomenon called mitotic catastrophe (leading to cancer remission), or mitosis exit in metaphase, also known as mitotic slippage, driving cancer progression [74-76]. The outcome is dictated by the balance between the opposing activities of the apoptotic machinery and two mitotic ubiquitin ligases. Slippage is thought to involve the gradual proteolysis of cyclin B1 by the APC/C and $CRL2^{ZYG11A/B}$ ubiquitin ligases [77-79]. How cancer cells treated with spindle poisons (e.g. taxol) avoid apoptosis during mitosis remains unclear. It has been suggested that polyploidy might confer a survival advantage on tumour cells, contributing to the evolution of treatment resistance [80]. Mitotic slippage events have also been described during colorectal cancer cell divisions in tumours with *adenomatous polyposis coli* (*APC*) gene mutations [81]. *APC* mutations are the most frequent type of mutation in human colorectal tumours [82]. APC is involved in the WNT signalling pathway, but it also binds to mitotic spindle microtubules, and its absence impairs kinetochore–microtubule interactions, leading to chromosomal instability (CIN) in APC-driven murine colon carcinomas [83]. Dikovskaya and co-workers have demonstrated that conditional knockout of *APC in vitro* and *in vivo* induces mitotic slippage that is linked to the genesis of a mononucleate tetraploid cell contingent [81]. However, the link between tetraploid cells with *APC* mutations and the increase in CIN in colon carcinogenesis remains unclear.

[H2] Cytokinesis failure

Cytokinesis is the final step in cell division and is initiated during anaphase [84]. It involves a finely regulated series of events to ensure that genomic and cytoplasmic materials are evenly distributed between the two nascent daughter cells. Four events must occur for the correct induction of cytokinesis: establishment of the division plane, contraction of the actomyosin ring, ingression of the cleavage furrow and, finally, formation of the intracellular bridge, the

degradation of which leads to cell abscission. Interference with the progression of these steps can lead to cytokinesis failure and to the genesis of binucleate polyploid cells (FIG. 2C) [85, 86]. Cytokinesis failure has been known for several decades to be a physiological process involved in the development of certain human tissues and organs, such as the heart [87], bone marrow [88] and liver [89]. For example, ventricular cardiomyocytes respond to the increase in blood flow after birth with an adaptive increase in volume (known as hypertrophy)[90]. This transition from hyperplasia to hypertrophy is clearly associated with polyploidization as it leads to the genesis of binucleate tetraploid cardiomyocytes[91]. The degree of polyploidization in ventricular cardiomyocytes differs between species and varies with age, but it might reach 85% in rodents and 30% in humans [91, 92, 93, 94]. Cyclin G1 has been identified as an important player in the molecular machinery that controls cardiomyocyte binucleation. The expression of cyclin G1 in neonatal cardiomyocytes promotes G1–S cell cycle transition but inhibits cytokinesis [95]. Cytokinesis failure in cardiomyocytes might also be caused by the abnormal localization of proteins that regulate the organization of the contractile ring (for example, Ras homolog gene family member A (RhoA), ROCK-I, ROCK-II and anillin) [87, 96, 97].

Cytokinesis failure has also been reported in a number of diseases, often in association with high rates of mutation or genomic instability [98-100]. In their elegant review, Lacroix and Maddox describe several examples of cytokinesis failure events in different diseases: Wiskott–Aldrich syndrome, X-linked neutropenia, Fanconi anaemia, Lowe syndrome, type II neurofibromatosis and age-related macular degeneration [101]. Interestingly, bulk chromatin, or even a single lagging chromosome trapped in the cleavage furrow, can induce cytokinesis failure and tetraploidization in human cells [102, 103]. Cytokinesis defects have also been reported in many different types of cancer cells (e.g. breast, colon and ovarian), as a result for example of dysfunctions of various proteins that control the cytokinesis process (aurora A, mitotic arrest-deficient 2 (MAD2) and large tumour suppressor 1 (LATS1)) [21].

[H1] Polyploidy in the liver

The liver is a vital organ with an extraordinary range of physiological functions, including the metabolism of carbohydrates, lipids and amino acids, detoxification of xenobiotic compounds, synthesis of serum proteins, and bile production and secretion. These functions are performed primarily by hepatocytes, which account for 70% of the cells of the human liver [104]. Interestingly, the metabolic functions of hepatocytes are specific to their position along the portocentral axis, making it possible to distinguish between 'periportal' hepatocytes (which are involved in gluconeogenesis, ureagenesis, β-oxidation of fatty acids, amino acid breakdown and cholesterol biosynthesis) and 'pericentral' hepatocytes (which are involved in glycolysis, *de novo* lipogenesis and alcohol detoxification) [105-109]. Another source of hepatocyte heterogeneity is differences in ploidy. At birth, all hepatocytes are diploid, with a single nucleus containing two copies of each chromosome [110, 111]. During postnatal growth, the liver parenchyma undergoes cellular changes characterized by gradual polyploidization, leading to the emergence of hepatocytes of several different ploidy classes (FIG. 3) [110, 111]. Hepatocyte ploidy depends on the DNA content of each nucleus (nuclear ploidy: diploid, tetraploid, octoploid, and so on) and on the number of nuclei per hepatocyte (cellular ploidy: mononucleate, binucleate) [110, 111]. For example, polyploid hepatocytes can be tetraploid (either binucleate with two diploid (2*n*) nuclei or mononucleate with a single tetraploid (4*n*) nucleus) or octoploid (either binucleate with two tetraploid (4*n*) nuclei or mononucleate with a single octoploid (8*n*) nucleus). The liver has long been known to display polyploidy, and various studies have determined the proportions of polyploid hepatocytes in the liver parenchyma of different species [112-116]. For example, up to 90% of adult hepatocytes are polyploid in rodents [89, 117-119], versus approximately 30% in humans [120-124]. An increase in cell size is the most obvious and consistent consequence of an increase in ploidy. Several studies have shown that a doubling of chromosome number approximately doubles the size of hepatocytes in humans

and mice parenchyma [125-128]. There is no substantial difference in the volume of a given state of ploidy, for example, between a tetraploid contingent of diploid binucleate cells (2×2*n*) and a tetraploid contingent of tetraploid mononucleate cells with a single tetraploid (4*n*) nucleus [127, 129]. Intriguingly, when the volume of the cell increases twofold during polyploidization, the surface area of cellular structures increases only 1.4-fold (e.g. yeast and mammalian cells) [99]. In addition, as has already been demonstrated in *Arabidopsis thaliana* [130], changes in the proportions of cellular elements upon polyploidization, as chromosome movement, might influence nuclear structure.

[H1] Polyploidy in liver development

Polyploidization during development has been analysed almost exclusively in rodent models. All hepatocytes in the newborn rodent liver are diploid and have high rates of proliferation [131]. However, cell division gradually declines in rodents, such that, by six weeks of age, DNA synthesis is detected in only a few hepatocytes, at rates similar to those reported for normal adult liver [89, 132]. Various *in vivo* studies have shown that polyploidization begins in the first few weeks after birth with the genesis of binucleate tetraploid hepatocytes (2×2*n*) [89, 119, 133-135]. During this period, diploid hepatocytes (2*n*) follow either a normal cell cycle or an adapted cell cycle with karyokinesis but no cytokinesis (cytokinesis failure; FIG. 3). Proliferating binucleate tetraploid hepatocytes can then repeat DNA replication, leading either to cytokinesis, which generates two mononucleate tetraploid hepatocytes (4*n*), or to another failure of cytokinesis, which generates a binucleate octoploid hepatocyte (2×4*n*) (FIG. 3) [89]. This situation results in the establishment of physiological polyploidy. Margall-Ducos et al. showed that the canonical cytokinesis programme is disrupted owing to the absence of actinomyosin ring formation [134]. Indeed, the organization of the actin cytoskeleton during the anaphase–telophase transition is impaired in these conditions. The microtubules fail to contact

the cortex, and the molecular signals essential for cleavage plane specification (for example, aurora B and polo-like kinase 1 (PLK1)) are therefore not correctly delivered. Consequently, active RhoA, the principal orchestrator of cytokinesis, does not concentrate at the division site, and the contractile ring is not formed [134]. During postnatal growth, cytokinesis failure events occur around the time of weaning [134] a period associated with profound changes in feeding behaviour, hormone concentrations and metabolic pathways [136]. Indeed, insulin has been identified as a key factor in the genesis of binucleate tetraploid hepatocytes [137, 138]. In rodent, impairment of insulin signalling greatly decreases the formation of binucleate progenies, whereas repeated insulin injections promote the generation of polyploid liver cells. The phosphoinositide 3-kinase (PI3K)–AKT pathway appears to be the principal downstream pathway that mediates the cellular effects of insulin in these conditions. Various studies have revealed that PI3K–AKT regulates actin cytoskeleton polarization and reorganization during processes such as cell migration and invasion [139]. The inhibition of AKT activity in dividing hepatocytes induces the correct reorganization of actin during mitosis and the correct localization of RhoA to the site of division, which is essential for successful cytokinesis [137, 138]. Other factors have also been shown to control binucleation during liver development. E2F transcription factors, which are master regulators of cell cycle progression [140], are also essential. This family includes activators (E2F1, E2F2 and E2F3), repressors (E2F4, E2F5 and E2F6) and two atypical repressors (E2F7 and E2F8). In the mouse liver, E2F1–E2F6 levels are low throughout life, whereas E2F7 and E2F8 levels are high during the first seven weeks after birth, a period that coincides with liver polyploidization [119]. *E2f7 and E2f8*-knockout mouse livers were highly enriched with diploid hepatocytes (60-70% of hepatocytes in KO versus 3-4% in control livers)[65, 119, 141]. A substantial portion of the deregulated transcripts identified in *E2f7 and E2f8*-knockout mouse livers were involved in cytokinesis (*Ect2*, *Mklp1*, *Racgap1*). Interestingly, during hepatocyte binucleation, Ect2, Mklp1 and

Racgap are impaired in relaying information from the spindle to the cortex, thereby preventing the formation of a functional contractile actomyosin ring and thus cytokinesis [134]. E2F transcription factors have already been identified as key factors in the polyploidization process in *A. thaliana* and *D. melanogaster* [142-144]. A similar phenomenon has been described for trophoblast giant cells [65] and endometrial stomal cells [145] in mice. Interestingly, microRNAs (miRNAs) are also drivers of physiological binucleation [133]. Mouse liver miRNAs are expressed in an age-dependent manner, and a subset of these molecules, miR-122 in particular, is differentially expressed during postnatal development. *Mir122* knockout livers displayed 60%-70% reduction in the number of polyploid hepatocytes, this defect being rescued by miR-122 overexpression [133]. During liver development, miR-122 directly antagonizes pro-cytokinesis targets, thereby regulating hepatocyte binucleation [133]. Finally, the silencing of cell cycle regulator genes, such as *Cdk1* [146], *Trp53* [147, 148], *Cdkn1a* [148, 149], *cMyc* [150], *Ccne* [151], *Birc5* (which encodes survivin) [152], *Ssu72* [153], *Stk3* (also known as *Mst1/2*) [154], *Tgfb1* [155] and *Rb1* [148, 156], has been shown to modify cellular or nuclear liver polyploidization in various mouse models. However, the roles of these diverse genes in controlling the cell cycle and division during liver development in relation to binucleation are unknown. Together, these studies highlight the essential contribution of cytokinesis failure to physiological liver polyploidization. Future studies should investigate how the combined activities of AKT, mTORC2, E2F7, E2F8 and miR-122 lead to inhibition of the cytokinesis machinery and the expansion of binucleate hepatocytes during liver development.

[H1] Polyploidy during regeneration after tissues injuries

The liver is continually exposed to tissue injuries and stresses throughout life. In the adult human liver parenchyma, hepatocytes are slowly replaced, with each cell having a mean lifespan of approximately 200–300 days [157], but they retain the ability to proliferate rapidly in

response to aggression. The induction of liver regeneration by partial hepatectomy is a model of compensatory hypertrophy, and several studies have revealed changes in ploidy profile during this process. All hepatocytes enter the cell cycle after partial hepatectomy. By the end of the regenerative process, cellular ploidy (binucleate polyploid hepatocytes) decreases, and nuclear ploidy (mononucleate polyploid populations) increases (FIG. 4) [158-162]. Interestingly, Wilkinson et al. demonstrated that the global ploidy spectrum remained unchanged after complete liver regeneration in the partial hepatectomy mouse model [141], which suggests that the decrease in cellular ploidy is compensated for by the increase in nuclear ploidy. Miyaoka et al. have used cell lineage approaches and a high-throughput imaging system to investigate the mechanisms controlling polyploidization during mouse liver regeneration [28]. They found that, although all hepatocytes entered into the cell cycle after partial hepatectomy, progressed into S phase, only about half continue through mitosis suggesting that some hepatocytes undergo endoreplication (amplification of nuclear ploidy). Importantly, hepatocytes that will go into mitosis undergo cell division (genesis of mononucleate progenies). Thus, by contrast to liver development, during liver regeneration, there is a shift from cellular polyploidy (polyploid hepatocytes, predominantly binucleate) to nuclear polyploidy (polyploid hepatocytes, predominantly mononucleate) (FIG. 4). Pathological conditions, such as hepatic metabolic overload (of iron or copper) [163-165], telomere attrition [166] and chronic viral infection (hepatitis B and C virus) [123, 124, 167], also promote liver polyploidization, by as yet uncharacterized mechanisms (FIG. 4). Polyploidy spectrum has been also characterized in nonalcoholic fatty liver disease (NAFLD) (FIG. 4) [31, 168]. In mice models of NAFLD, there was an enrichment of highly polyploid mononucleate hepatocytes in the fatty liver parenchyma. The same phenotype was observed in patients with nonalcoholic steatohepatitis. The key question is, how are these cells generated? The cell cycles of normal hepatocytes were compared with fatty hepatocytes in mouse primary cultures. Fatty hepatocytes

progressed through G1 phase and entered S phase, but their progression through the S and G2 phases was delayed relative to control cells. This delay was associated with the activation of a G2–M 'DNA damage signal' (ATR-p53-p21 pathway) that precludes the activation of the mitotic kinase (CDK1–cyclin B) and leads to endoreplication [31, 168]. This work led to the identification of oxidative stress as a key driver of pathological polyploidization in the livers of patients with NAFLD. These findings provided the first demonstration of the generation, by endoreplication, of pathological polyploid hepatocytes in a damaged liver, through activation of a DNA damage-signalling axis. It is tempting to speculate that endoreplication is a favoured mode of liver tissue repair, but further studies are needed to determine how various genotoxic stresses and cell cycle regulators act together to promote endoreplication, and also the fate of the polyploid hepatocytes generated in such conditions. Emerging data from multiple experimental models have suggested a conserved role for endoreplication as an alternative means of proliferation in a context of genome instability [57, 67].

[H1] Effects of polyploidy on liver function

Is hepatocyte polyploidization merely a manifestation of the physiological growth or aggression of liver tissue, or do polyploid cells have functions that are physiologically relevant? The biological importance of liver polyploidization remains unclear, but various hypotheses have been proposed, some of which have already been partially validated.

[H2] Polyploidization stage and terminal differentiation

Pioneering studies in rodent and human have shown liver polyploidy to be related to terminal differentiation and senescence [122, 131, 160, 161, 169-171]. As a result, many studies have tried to determine whether a polyploid hepatocyte can still divide in response to different proliferation stimuli. Surprisingly, several groups argued that polyploid hepatocytes seem not to be engaged in senescence but by contrast maintained a high rate of proliferation. For instance,

polyploid hepatocytes have been shown to divide as much as the diploid contingent after partial hepatectomy [28, 172]. The livers of *E2f8*$^{-/-}$ mice, which consist predominantly of diploid hepatocytes, have a regenerative capacity similar to that of wild-type livers, which have a large proportion of polyploid cells [119]. Similarly, in mouse models of chronic liver injury followed by competitive repopulation of hepatocytes, diploid and octoploid hepatocytes have been shown to have equivalent repopulation potentials [117, 173]. By contrast, other studies have supported the idea that the polyploid state acts to suppress growth and restrict proliferation. Ganem et al. reported that cytokinesis failure during postnatal murine liver development triggers the Hippo–LATS2–p53 pathway, which restricts the growth of tetraploid hepatocytes [174]. In addition, tetraploid murine hepatocyte growth has been shown to increase in a *Trp53*$^{-/-}$ [147, 174]. In 2018, Wilkinson et al. investigated whether diploid hepatocytes from wild-type mouse livers had a proliferative advantage compared with polyploidy contingent [141]. They observed, both *in vitro* (primary mouse hepatocyte cultures) and *in vivo* (mouse liver regeneration assays), similar responses of diploid and polyploid hepatocytes to hepatic mitogens, indicating that proliferation kinetics are unrelated to differential responses to growth stimuli. However, they also showed that diploid hepatocytes entered and completed the cell cycle more rapidly than their polyploid counterparts. Differences were also observed among polyploid hepatocytes, with tetraploid cells entering the cell cycle more rapidly than octoploids. The authors suggested that cell cycle regulation might subtly differ between diploid and polyploid contingents (for example, early replication licensing and mitosis progression). The restrictive proliferation of the polyploid contingent might be related solely to ageing. Diploid, tetraploid and octoploid hepatocytes from two-month-old mice have identical capacities to repopulate the liver and do not express specific senescence markers, such as p16, p21 and p53 [175, 176]. However, as mice age, the expression of these senescence markers becomes substantially stronger in octoploid than in diploid and tetraploid

hepatocytes, and the repopulation potential of the octoploid contingent also decreases [175, 176]. Collectively, these data suggest that polyploid hepatocytes retain the ability to respond to mitogen stimuli and to proliferate. However, during ageing, and possibly in some liver diseases, polyploidy might induce senescence-type changes. Studies are therefore required to improve our understanding of ploidy-specific proliferation in different liver injuries such as chronic liver disease.

[H2] Polyploidization as an enhancer of liver functions

Polyploidy is a common strategy for organogenesis in various systems and organs including the liver and, over the past 10 years, many studies in different experimental models have highlighted specific functions for polyploid cells [7, 24, 25, 177]. An innovative study by Rios and co-workers revealed a role for specific mammalian polyploid cells [178]. They observed binucleate polyploid cells in the lactating mammary glands of several different species (e.g. humans, cows and mice) [178]. In mice, aurora kinase A (AURKA) and PLK1 control the generation of these binucleate cells by cytokinesis failure at the time of the switch to lactation in response to various signals, including prolactin and epidermal growth factor. The binucleate alveolar cells were shown to be essential for lactation. Deletion of *Aurka* inhibited the formation of binucleate cells and decreases milk synthesis [178].

The liver participates in diverse functions, and it is tempting to speculate that physiologically polyploid hepatocytes have their own features and roles. The gene expression profile in the liver changes throughout the lobule (along the portocentral axis), resulting in a spatial zonation of tasks [105-109]. Several groups have investigated whether polyploid hepatocytes display a specific distribution within mouse hepatic lobules that might account for specific functions, but the findings of these studies are conflicting. Some studies suggested that the periportal region contains fewer polyploid hepatocytes than the perivenous region [118, 171], whereas others suggested that the proportions of polyploid cells were similar in both regions

[119, 134]. Two studies used tissue-imaging systems to reconstruct spatial zonation in the murine liver and to quantify hepatocyte polyploidy [129, 179]. They showed that the periportal and perivenous regions were enriched in tetraploid hepatocytes but had a low density of octoploid hepatocytes, whereas the mid-lobule region (equidistant between the periportal and perivenous regions) displayed the opposite pattern: it was enriched in octoploid hepatocytes but had a low density of tetraploid hepatocytes. They concluded that liver polyploidy proceeds in spatial waves, advancing more rapidly in the mid-lobule region than in the periportal and perivenous zones. One study investigated whether this zonation of polyploidy was conserved in normal human hepatic lobules. Bou-Nader et al. observed that neither mononucleate ($4n$ or $\geq 8n$) nor binucleate ($2\times 2n$ or $\geq 2\times 4n$) polyploid hepatocytes had a specific zonal distribution [120]. Much less is known about metabolic zonation in the human liver than in the mouse liver [108]. Single-cell *in situ* experiments will be required to analyse the combined effects of metabolic zonation and polyploidy on hepatocyte fate.

A simple model used to explain the effects of polyploidy on cellular functions increases transcription/traduction on a per cell basis (e.g. twice as many genes would produce twice as many proteins) [177, 180, 181]. However, the situation is not quite that simple, as it has been shown, in a number of species (e.g. plants, *Drosophila*, mammalian cells) that polyploidy promotes nonuniform genome, transcriptome, epigenome and metabolome modifications [177]. A few years ago, Lu et al. used a microarray approach to analyse the gene expression profile of fluorescence-activated cell sorting (FACS)-isolated diploid, tetraploid and octoploid hepatocytes from wild-type mice [182]. Surprisingly, only a few differences in gene expression profile between the different populations were found. Kreutz and co-workers suggested that gene expression profiles differ between diploid and mononucleate polyploid hepatocytes, but not between diploid and binucleate polyploid hepatocytes [183]. This finding would suggest that increases in nuclear ploidy in the liver parenchyma affect hepatocyte fate, a hypothesis that

was tested in mice that lack the mitotic kinase CDK1. Following partial hepatectomy, *Cdk1*-knockout livers regenerate fully, but hepatocytes endoreplicate, resulting in a large proportion of mononucleate polyploid hepatocytes in the liver [146]. Using this model, Miettinen et al. showed that higher ploidy levels were associated with lower levels of expression of mitochondrial and *de novo* lipid biosynthesis genes and with higher levels of expression of glycolysis genes [181]. The inhibition of mitochondrial functions and lipid synthesis increases cell size in human epithelial cells, suggesting a possible causal link cellular metabolism and polyploidy [181]. These results are consistent with the results of a comparative genome-scale analysis of mouse and human liver tissues with different levels of polyploidy, in which highly polyploid livers displayed anaerobic energy production, with ATP obtained from carbohydrates rather than from fatty acids [184, 185]. Collectively, these results suggest a probable link between polyploidy and energy demands. Cells that display endoreplication or cytokinesis failure can channel the energy that would have otherwise gone into cell division (in particular, ATP and lipid membrane consumption) for other purposes [99]. Thus, polyploidization might facilitate rapid adaptation to stresses and new–environmental cues. Finally, Itzkovitz and collaborators demonstrated that liver polyploidy dampens the intrinsic variability associated with transcriptional bursts [186]. In prokaryotes and eukaryotes, many genes are transcribed in bursts, with stochastic production of mRNA from the transcription sites, and with transcription switching between 'on' and 'off' states [187]. Using single molecule fluorescence *in situ* hybridization (FISH), the authors demonstrated that gene expression in the intact mouse liver consists of transcriptional bursts [186]. They observed that transcriptional noise tends to be lower in tetraploid hepatocytes than in diploid hepatocytes. The authors suggest that liver polyploidization might therefore counteract the noise increase caused by genes becoming 'burstier' with age, leading to a more controlled gene expression.

[H2] Polyploidization creates genetic diversity

Proliferating polyploid hepatocytes can increase their DNA content, but they can also reduce it, through a process known as ploidy reversal [117, 121, 188]. Polyploid hepatocytes have larger numbers of chromosomes than their diploid counterparts, but they also have supernumerary centrosomes. For example, tetraploid hepatocytes (mononucleate or binucleate) have two centrosomes in G1–S phases and four centrosomes in G2–M phases [89]. In most cases, proliferating polyploid hepatocytes form a bipolar mitotic spindle through a specific centrosome cluster (two centrosomes at each spindle pole), leading to the generation of polyploid progenies (FIG. 4) [89, 117]. Polyploid hepatocytes can also form multipolar mitotic spindles and, in this case, cell division can lead to the generation of three or more daughter cells with lower ploidy states than maternal cells (a phenomenon known as ploidy reversal) (FIG. 4)[117]. The formation of a multipolar mitotic spindle is also linked to chromosome segregation errors that arise from merotelic chromosome attachments (the attachment of one kinetochore to both mitotic spindle poles). In this case, the division of polyploid hepatocytes leads to random whole-chromosome gains and/or losses, resulting in aneuploid contingents [117, 121]. The connection between polyploidy, ploidy reversal and aneuploidy has been called the 'ploidy conveyor' [110]. Interestingly, aneuploidy is observed in many different healthy human tissues, including those of the skin, brain and placenta [117, 121, 188-191]. However, the degree of aneuploidy in the healthy liver remains a matter of debate. Studies of proliferating polyploid hepatocytes in primary mouse culture have revealed high rates of chromosome segregation errors and aneuploidy (approximately 60%) [117, 192]. By contrast, single-cell sequencing of hepatocytes in mouse and human liver tissues has revealed only a low level of aneuploidy (about 5%) [188]. An elegant study by Knouse and collaborators examine the importance of the tissue environment for chromosome segregation fidelity in the polyploid liver [172]. By comparing images of mitotic polyploid hepatocytes in mouse regenerating liver

or in primary cultures, they demonstrated that tissue architecture is particularly important for the correction of merotelic attachments and, therefore, for chromosome segregation fidelity in the polyploid liver [172]. Recently, Marcus Grompe's group has developed a sophisticated multicolor reporter allele system to genetically label and trace *in situ* mouse polyploid hepatocytes *in situ* [193]. They observed that polyploid hepatocytes regenerate injured livers and frequently reduce their ploidy. Ploidy-reduced progenies proliferate and keep the property to increase their ploidy content. Importantly, they observed that during ploidy reduction chromosome segregation is not random but faithful. All these data reinforce our view that the ploidy conveyor model is intriguing, not least because it could generate genetic diversity in the healthy liver. Duncan and co-workers have analysed the role of hepatic aneuploidy in chronic liver injury, using $Fah^{-/-}$ mice, a well-known model of hereditary tyrosinaemia type 1 [194]. In response to massive liver failure, hepatocytes with a partial loss of chromosome 16 expanded, repopulated the liver and restored liver function [192]. The partial loss of chromosome 16, which carries the gene that encodes homogentisate 1, 2-dioxygenase, confers resistance to the disease in this model of liver injury. Interestingly, a loss of polyploidy in the liver (*E2f7*/*E2f8* double knockout mouse model) resulted in decreased aneuploidy rates and decreased the ability of the liver to adapt to tyrosinaemia-induced liver failure [195]. Further studies are now required to determine whether ploidy reversal and specific aneuploid hepatocytes can facilitate adaptation in chronic human liver diseases.

[H2] Benefits of polyploidization for tissue repair

Polyploidy can be benefic after acute organ failure in different organs, as shown in *Drosophila* in particular. Losick and co-workers have shown that polyploidization contributes to wound healing in adult *D. melanogaster* epithelia [196]. Indeed, the epithelial cells that surround the injury site re-enter S phase, but, rather than dividing, these cells undergo endocycling and become polyploid. In this case, epidermal repair is inhibited when

polyploidy is blocked. In mammals, polyploidy can also be beneficial for tissue repair (e.g. mouse, human) [24, 25, 197]. In an elegant study, Cao and co-workers devised genetic tools and live imaging methods for visualizing cell-cycle dynamics during the regeneration of the zebrafish epicardium [26]. Their results demonstrate that cells in the leading edge (called 'leader cells') of the regenerating epicardium undergo endoreplication or cytokinesis failure and generate polyploid cells. Farther from the lesion, 'follower cells' complete the cell cycle. An increase in mechanical tension at the leading edge was found to be a key factor driving polyploidy. Interestingly, most polyploid leader cells undergo apoptosis when regeneration is complete. These results suggest that mechanical tension within a tissue can favour polyploidy to support tissue regeneration[26].

As the liver is the most robust regenerative organ in the human body and one of the best examples of polyploid tissues, it was tempting to speculate that the polyploid contingent has a major role in tissue repair. As described above, polyploid hepatocytes proliferate during mouse liver regeneration after partial hepatectomy. Surprisingly, these cells being as effective as diploid hepatocytes at restoring injured tissues [28]. This phenomenon has been analysed in the liver of *E2f7*/*E2f8* double knockout (LDKO) mice presenting reduced polyploidy. Resected polyploid livers (wild-type) support regeneration as effectively as resected predominantly diploid livers (LDKO) [119, 198]. Furthermore, no difference in regeneration was observed when wild-type and LDKO livers were treated acutely with hepatotoxins, such as carbon tetrachloride or 3,5-diethoxycarnonyl-1,4-dihydrocollidine [65, 119]. These results suggest that the restoration of liver size by polyploid cell growth during liver regeneration after partial hepatectomy or after acute injury is not beneficial. Future studies will determine whether polyploidization is beneficial for tissue repair following chronic liver injury.

[H1] Polyploidy in liver tumorigenesis

Polyploid cells have been observed in multiple cancer types such as pancreatic [199], cervical [53], lung [200], colon [201] and prostate [202]. Genomic studies have revealed that approximately 30% of solid tumours have polyploid or near-polyploid karyotypes [36, 203]. Clinically, polyploidy has been associated with aggressive, difficult-to-treat different solid tumours (e.g. colorectal and pancreatic cancers) [80, 203, 204]. Numerous studies have observed that polyploid cells are present from the transition from premalignant to malignant disease suggesting that a genome-multiplying event can be a driver of tumorigenesis [53, 205, 206]. It is therefore not surprising that mechanisms have evolved to limit the proliferation of these cells. It is well illustrated that the p53 pathway limits the proliferation of polyploid cells to protect genomic integrity [30, 207, 208]. Polyploid cancer cells (e.g. colorectal, fibrosarcoma) can be also recognized by the immune system in such a way that their growth is suppressed or delayed [74, 209]. It has been demonstrated that $CD4^+$ and $CD8^+$ T lymphocytes, the interferon system [210] and NK cells [211] are involved in the control of polyploid cells. One of the mechanism through which polyploid cells can be recognized by the immune system implies an endoplasmic reticulum (ER) stress response resulting in the exposure of the 'eat-me' signal calreticulin (CALR) at the cell surface [210, 212]. Senovilla and collaborators also demonstrated that polyploid mouse colon cancer readily proliferated in immunodeficient mice and conserved their increased DNA content [210]. Interestingly, the same cells injected into immunocompetent mice generated tumours only after a delay, and such tumours exhibited reduced DNA content, endoplasmic reticulum stress and calreticulin exposure [210].

The accepted model is that tetraploidy drives carcinogenesis by acting as a stochastic generator of genomic instability. As described earlier, polyploid cells have supernumerary centrosomes, and this feature is linked to high rates of chromosome missegregation in mitosis [207, 213, 214]. Also, one study neatly showed in colorectal cancer cell lines that tetraploidy induces replication stress through a massive deregulation of genes that are involved in the

correct functioning of the DNA replication machinery [215]. Replication stress has emerged as a source of CIN [216]. Several laboratories working on different experimental models have shown that polyploidy is a gateway state that participate to tumorigenesis [34, 70, 204, 217]. The pioneering experiments were performed by David Pellman and colleagues who showed that *TP53*$^{-/-}$ tetraploid mammary epithelial cells gave rise to malignant tumours in nude mice, whereas *TP53*$^{-/-}$ diploid mammary epithelial cells did not [34, 217]. This finding led to the suggestion that tetraploidy causes tumorigenesis. The temporal relationship between *TP53* mutations and polyploidy has been analysed in tumours from prospectively characterized patients with advanced cancers (e.g. colorectal, pancreatic, lung, uterine) [203]. As expected, polyploidy was statistically associated with *TP53*-mutated tumours. Interestingly, telomerase reverse transcriptase (TERT) promoter mutations are associated with an E2F-mediated G1 arrest defect, but not with polyploidization [203]. Overall, these data suggest that polyploidization is a key step in tumour formation and progression.

The link between polyploidy and the origin of cancer has primarily been demonstrated in tissues that are physiologically diploid. The role of polyploidy during liver tumorigenesis is quite puzzling, owing to the physiologically polyploid state of normal adult liver. Whether polyploidy is a risk factor for or protective against malignant transformation was until recently unknown. The most widely-accepted hypothesis is that liver polyploidy acts as a gatekeeper of tumorigenesis [218]. In theory, physiological polyploidization could protect cells against genotoxic damage by increasing gene copy numbers per cell, which could at least buffer gene-inactivating mutations [22, 177]. We can easily imagine that in a diploid cell the loss of heterozygosity of a tumour suppressor gene could have dramatic consequences, such as uncontrolled proliferation. Acquiring multiple sets of chromosomes could provide backup tumour suppressor gene copies. In a series of elegant experiments, Zhang et al. demonstrated that polyploid mouse livers were protected from tumour-suppressor loss of heterozygosity [198].

To modify the liver ploidy spectrum, two genes were silenced: *Anln*, an actin-binding protein required for cytokinesis, and *E2f8*, which is required for liver polyploidization. Ploidy was substantially increased after *Anln* knockdown (livers with the majority of hepatocytes are polyploid) and decreased after *E2f8* knockdown (livers with the majority of hepatocytes are diploid). The authors observed that higher levels of polyploidy reduced tumour incidence in diverse mouse liver cancer models, suggesting that polyploidy helps to prevent tumour development. Notably, in this study, ploidy content has been modified in non-tumoural liver tissue with wild-type *Trp53*. As demonstrated in others contexts, it is likely that polyploidy in the absence of *Trp53* could be a driver of liver tumorigenesis. The presence of polyploid cells within liver tumours could also act as a driver of tumour progression. In 2019, an *in situ* imaging approach was developed to determine whether cellular and nuclear ploidy content is altered during human liver tumorigenesis [120]. The ploidy spectra of surgically resected tissues from patients with hepatocellular carcinoma (HCC) as well as from healthy controls were determined. These data show that the binucleate polyploid fraction, or cellular ploidy, is drastically reduced during human liver tumorigenesis (≈15% in normal tissue versus 5% in tumoral tissue). Conversely, the mononucleate polyploid fraction, or nuclear ploidy, is amplified in HCCs (≈12% in normal tissue versus 33% in tumoral tissue). Next, Bou-Nader et al. compared nuclear ploidy spectra on the basis of histological and molecular features of HCC tumours. Nuclear ploidy was sufficient to distinguish between premalignant and malignant liver parenchyma. Mononucleate polyploid hepatocytes were enriched in HCCs that harboured a low grade of differentiation, a high proliferation rate and a poor prognosis. Furthermore, it was observed that *TP53* mutations account for a higher percentage of mononucleate polyploid hepatocytes than both *TERT* promoter-mutated and *CTNNB1*-mutated HCCs.

Taken together, these results indicate that polyploid hepatocytes act as a kind of 'Jekyll and Hyde' during liver tumorigenesis (FIG. 5). A polyploid state could provide protection from tumorigenesis by providing extra copies of tumour suppressor genes and by restricting hepatocyte proliferation in pre-malignant tissue with wild-type *TP53*. Conversely, amplification of mononucleate polyploid hepatocytes within HCCs is associated with *TP53* mutations, a high proliferative rate and a poor prognosis.

[H1] Conclusions

Polyploidization is a fascinating mechanism that is essential for tissue homeostasis but also has a role in tumorigenesis. Studies have revealed that the liver is one of the few mammalian organs to display changes in ploidy in various circumstances. In physiological conditions, progressive polyploidization occurs during liver development and in ageing, indicating terminal differentiation. We have learned much about the generation of polyploid hepatocytes through cytokinesis failure, but we still have much to learn about the function of these cells in liver homeostasis.

There are still important questions that need to be addressed. Is there a difference in cellular states between diploid and polyploid hepatocytes (that is, in epigenetics, transcriptomes, proteomes, and so on)? Is there a limit to hepatocyte ploidy? How do polyploid hepatocytes cope with genome copy number variation and centrosome amplification? And finally, what are the molecular cues of the ploidy conveyor, and does liver aneuploidy affect human health? The liver parenchyma displays changes in ploidy following tissue injury or stress, particularly in the context of NAFLD. Future studies should aim to understand whether pathological polyploidization takes place in other pathologies, and to determine how these polyploid contingents behave in damaged livers. Do they have specific lobule zonation? Do they influence disease progression? It will be particularly interesting to determine whether

hepatocyte polyploidization affects the process of liver infection (for example, of HCV and HBV). Interestingly, *Plasmodium* parasites have been shown to preferentially infect and develop in polyploid hepatocytes [219].

Finally, a major challenge in this field will be to determine whether polyploid HCCs derive from polyploid or diploid hepatocytes. If they are derived from polyploid hepatocytes, then the use of polyploidization to protect the liver would be dangerous. Conversely, if polyploid tumours arise from diploid hepatocytes, then the polyploid state might provide protection in pathological settings by providing extra copies of tumour suppressor genes and restricting hepatocyte proliferation.

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Fig. 1 | Characteristics of polyploidy. a| Ploidy is the number of complete sets of chromosomes in a cell. Somatic individual organisms, tissues and cells can be described according to the number of sets of chromosomes present: haploid (1 set), diploid (2 sets), tetraploid (4 sets). **b** | Polyploidy is defined on the basis of the DNA content of each nucleus, which is called nuclear ploidy (for example, a cell can be tetraploid mononucleate (4*n*) or octoploid mononucleate (8*n*)), and on the number of nuclei per cell, which is called cellular ploidy (for example, a cell can be tetraploid binucleate (2×2*n*) or hexaploid trinucleate (3×2*n*)). *n*, chromosome number.

Fig. 2 | Molecular mechanisms that lead to the polyploid state. Polyploid cells can be generated through various mechanisms that are classified according to their dependence on the cell cycle. **a** | The only cell cycle-independent process that leads to polyploid cells is cell fusion, which can occur during virus-mediated infections or through receptor–ligand interactions, giving rise to binucleate or multinucleated cells. **b** | The other mechanism relies on modifications of the canonical eukaryotic cell cycle that arise either during interphase (G1, S and G2) or mitosis (prophase, metaphase, anaphase and telophase). **c** | Endoreplication encompasses both endocycling and endomitosis. (1) Endocycling is comprised of alternating G and S phases, which eventually results in a rise in nuclear ploidy. This aberrant cell cycle is achieved by the combination of S-phase cyclin-dependent kinase (S-CDK) oscillation and mitotic entry impediment, either through cyclin B proteolysis or M-phase cyclin-dependent kinase (M-CDK) inhibition. (2) Endomitosis stems from issues that arise during mitosis. For instance, prolonged spindle assembly checkpoint (SAC) activation in metaphase resulting from a non-amphitelic chromosome attachment can be accompanied by progressive cyclin B1 degradation. This process, known as mitotic slippage, allows the cell to escape mitosis and reach G1. (3) Finally, cytokinesis failure can result in the genesis of binucleate cells by

preventing the physical separation of the cytoplasm (through cytokinesis) in telophase. Cytokinesis failure can be induced as a result of any interferences emerging during the highly regulated process of cytokinesis. Lightning bolts indicate the location of cell-cycle anomalies, such as lagging chromosomes or cytoskeleton disorganization.

Fig. 3 | Polyploidization during post-natal liver growth.

a | Polyploidization of hepatic tissue is a progressive developmental process that takes place around the time of weaning and is regulated by insulin, PI3K–mTORC2, E2F and miR-122 signalling. At birth, hepatocytes are exclusively diploid mononucleate ($2n$). During post-natal liver development after weaning, diploid hepatocytes can either enter the normal cell cycle (indicated by the blue arrow), giving rise to two diploid hepatocytes, or follow an aberrant cell cycle that is characterized by incomplete cytokinesis (indicated by the orange arrow), giving rise to one binucleate tetraploid ($2\times2n$) hepatocyte. Progressive polyploidization takes place via this process in the liver parenchyma, and tetraploid and octoploid cells with one or two nuclei are formed. Up to 90% of adult hepatocytes in rodents and approximately 30% in humans are polyploid. **b** | During the complete cytokinesis process (left panel), the cytokinetic machinery (MgcRacGAP; red; left upper panel) localized during anaphase both on the central spindle and on astral equatorial microtubules and during telophase at the midbody. Consequently, RhoA (green; left lower panel), the major cytokinesis orchestrator, accumulates at the equatorial cortex in early telophase, leading to the formation of the cytokinetic ring and the genesis of two diploid progenies. During incomplete cytokinesis (right panel), microtubules are disorganized (green; right upper panel). Consequently, MgcRacGAP (red; right upper panel) is observed on the remaining interdigitating microtubules in anaphase and telophase but is never localized on unattached astral equatorial microtubules, indicating that molecular signals delivered by microtubules to the equatorial cortex are impaired. Furthermore, RhoA (green; right lower panel) does not correctly localize

at the equatorial cortex and is instead observed near the cell centre, close to central spindle microtubules. Consequently, activation of RhoA GTPase in the central cortex is impaired, leading to the genesis of binucleate progeny. *Adapted from Figure 6* [134].

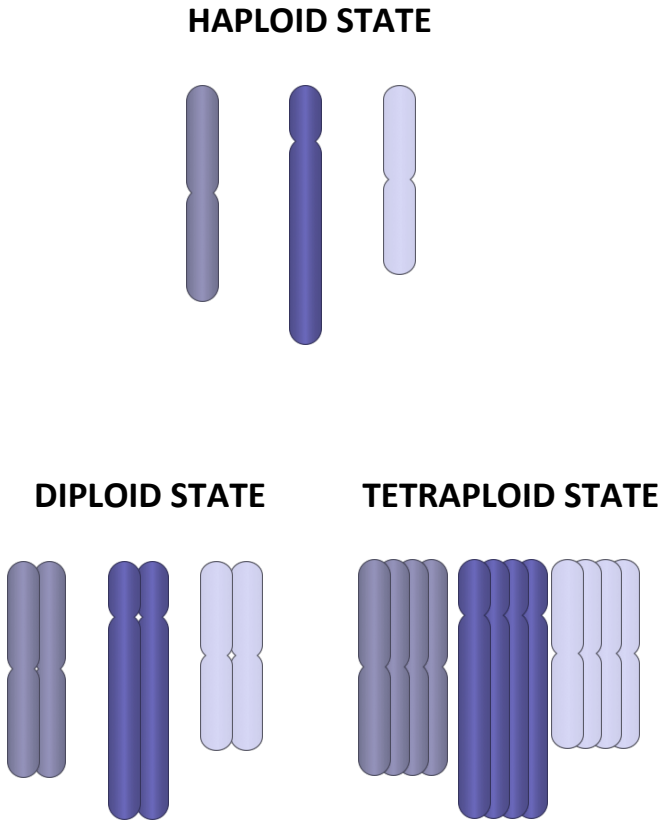
Fig. 4 | The genesis of mononucleate polyploid hepatocytes in physiopathological contexts. In human and mouse models of nonalcoholic fatty liver disease, the oxidative stress caused by an increase in levels of reactive oxygen species (ROS) induces the activation of the DNA damage response (ATR–p53–p21). This activation leads to multiple endoreplication cycles, generating polyploid mononucleate hepatocytes (for example, a tetraploid mononucleate cell (4*n*) or an octoploid mononucleate cell (8*n*)). During liver regeneration, there is also a shift from cellular ploidy to nuclear ploidy (for example, a diploid binucleate cell can form two tetraploid mononucleate cells, and a tetraploid binucleate cell can form two octoploid mononucleate cells or a 16*n* mononucleate cell). The hepatic parenchyma is also enriched in polyploid mononucleate hepatocytes (for example, 4*n* and 8*n* cells) following an iron or copper overload. *n*, chromosome number.

Fig. 5 | The polyploid state: gatekeeper or driver of liver tumorigenesis? Depending on the context and especially on the *TP53* status (that is, mutated or wild-type), the polyploid state can be regarded as a gatekeeper or a driver of hepatocarcinogenesis. Indeed, polyploid hepatocytes (for example, 4*n* and 8*n*) are more resistant to oncogenic events than diploid hepatocytes because they possess extra copies of tumour suppressor genes, which confers protection against loss of heterozygosity by buffering the effects of inactivating mutations. Furthermore, the polyploid state can hinder hepatocyte proliferation by means of the p53-dependent ‘tetraploid checkpoint’, which restricts proliferation and favours commitment to

cell death. It is therefore thought that liver tumours stem from diploid hepatocytes. *TP53* mutation represents a crucial event in the development of polyploid tumors, which are associated with enhanced aggressiveness and poor prognosis. Indeed, interfering with the p53 pathway allows polyploid cells to resume the cell cycle despite exhibiting deleterious chromosomal instability, which ultimately leads to aneuploidy. Hence, the polyploid state acts as a tumour-promoting mechanism by amplifying genomic aberrations and exacerbating tumour aggressiveness. However, it remains unknown whether polyploid tumours arise from a proliferating polyploid clone or from an initiating diploid tumour that has subsequently undergone polyploidization.

Figure 1:

A


HAPLOID STATE
DIPLOID STATE
TETRAPLOID STATE

B

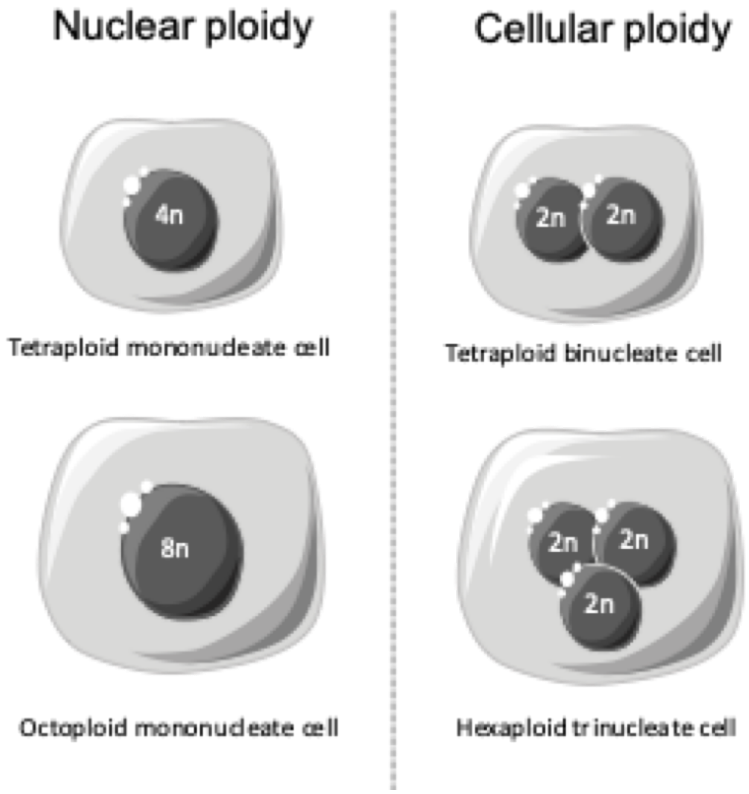

Nuclear ploidy
Cellular ploidy
4n
2n
2n
Tetraploid mononucleate cell
Tetraploid binucleate cell
8n
2n
2n
2n
Octoploid mononucleate cell
Hexaploid trinucleate cell

Figure 2:

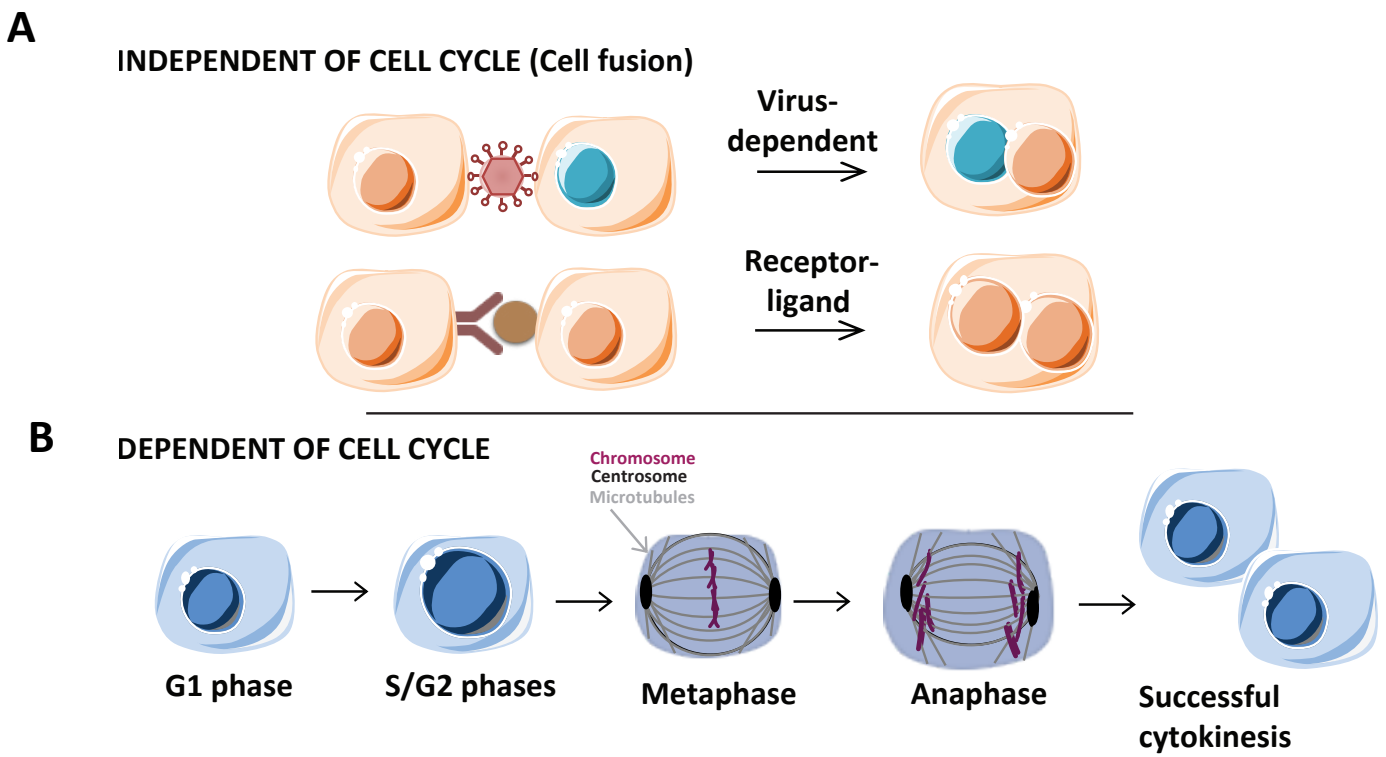

A
INDEPENDENT OF CELL CYCLE (Cell fusion)
Virus-dependent
Receptor-ligand
B
DEPENDENT OF CELL CYCLE
Chromosome
Centrosome
Microtubules
G1 phase
S/G2 phases
Metaphase
Anaphase
Successful cytokinesis

Figure 2:

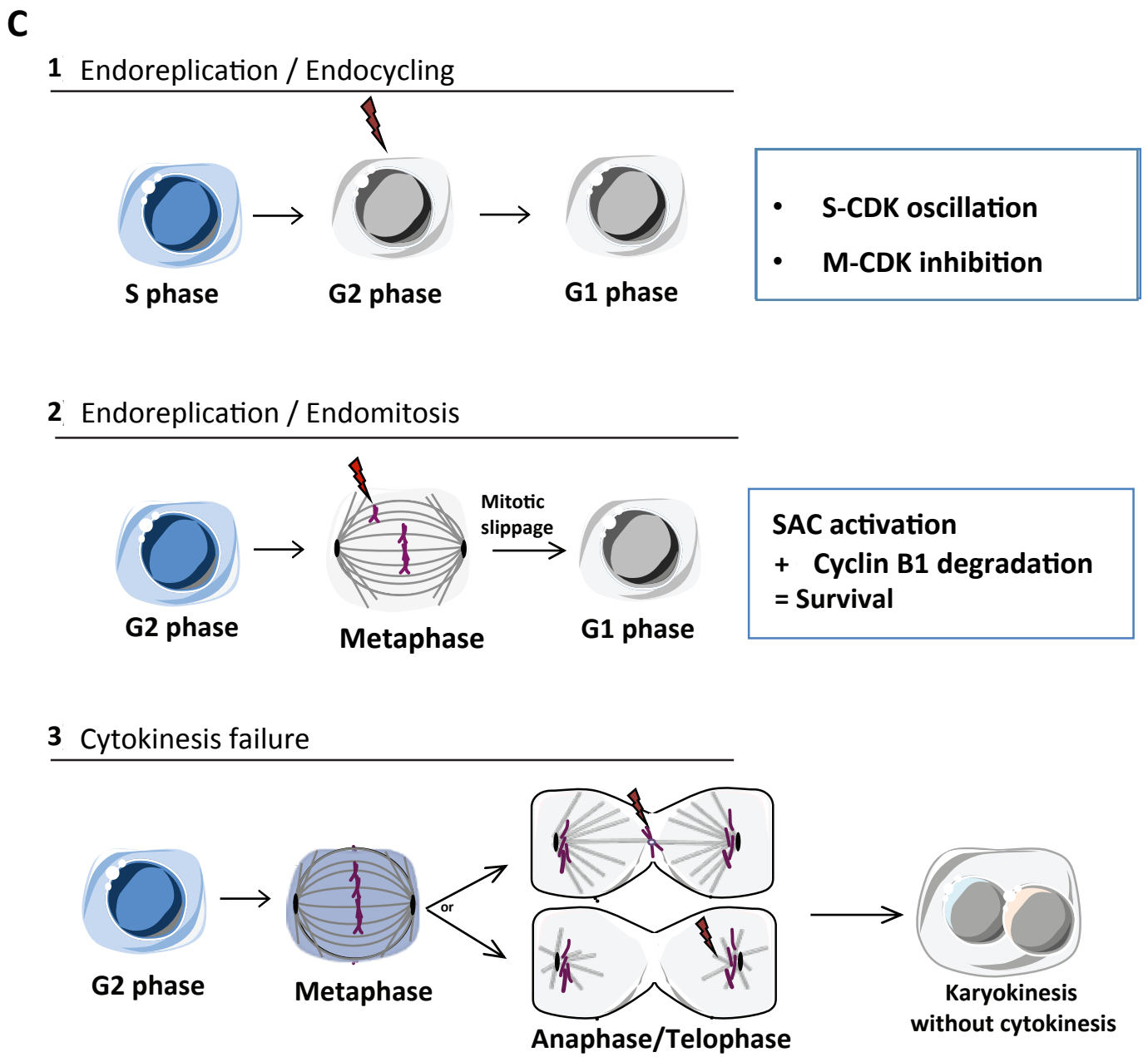

C
1 Endoreplication / Endocycling
S phase
G2 phase
G1 phase
• S-CDK oscillation
• M-CDK inhibition
2 Endoreplication / Endomitosis
G2 phase
Metaphase
Mitotic slippage
G1 phase
SAC activation
+ Cyclin B1 degradation
= Survival
3 Cytokinesis failure
G2 phase
Metaphase
or
Anaphase/Telophase
Karyokinesis without cytokinesis

Figure 3:

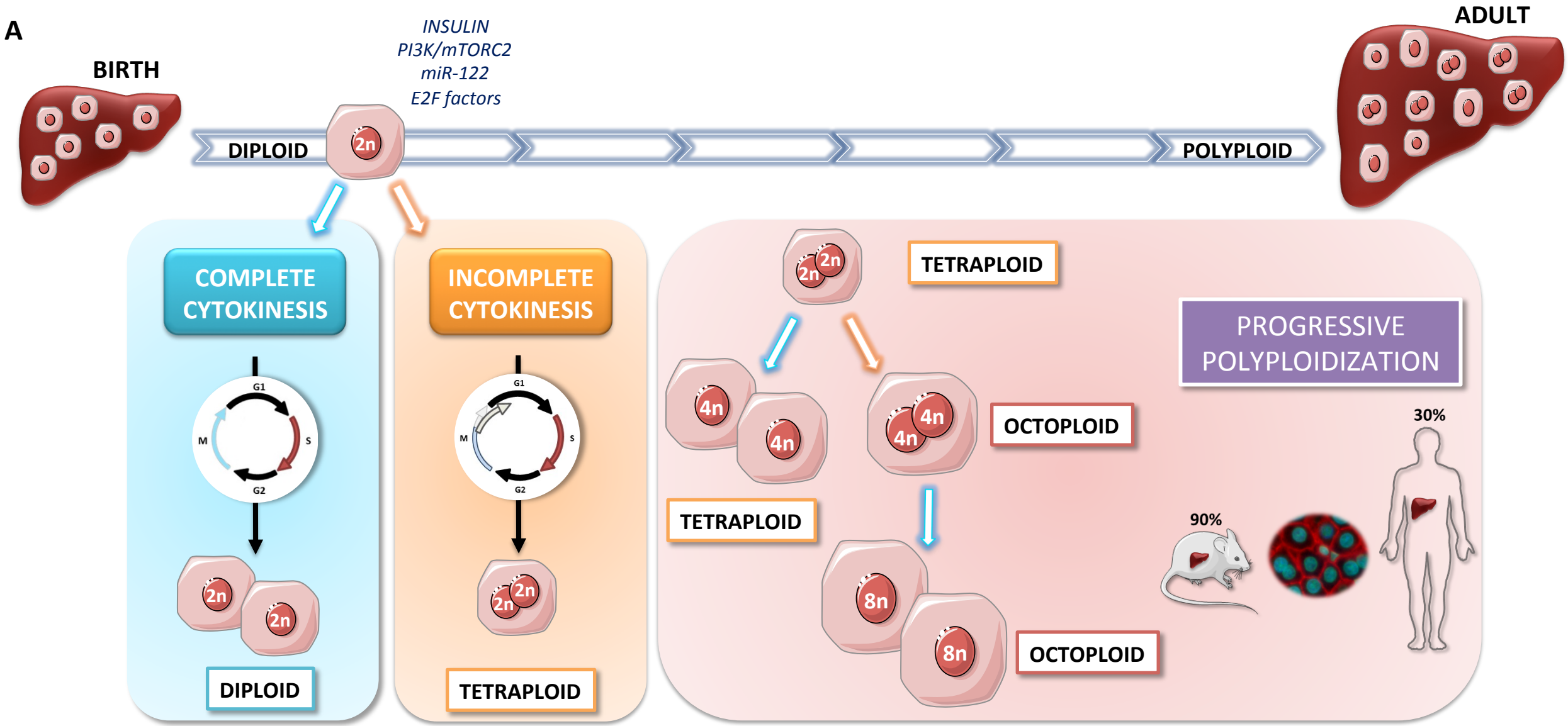

A
BIRTH
INSULIN
PI3K/mTORC2
miR-122
E2F factors
ADULT
DIPLOID
2n
POLYPLOID
COMPLETE CYTOKINESIS
INCOMPLETE CYTOKINESIS
G1
S
G2
M
2n
DIPLOID
TETRAPLOID
4n
TETRAPLOID
OCTOPLOID
8n
OCTOPLOID
PROGRESSIVE POLYPLOIDIZATION
30%
90%

Figure 3:

B

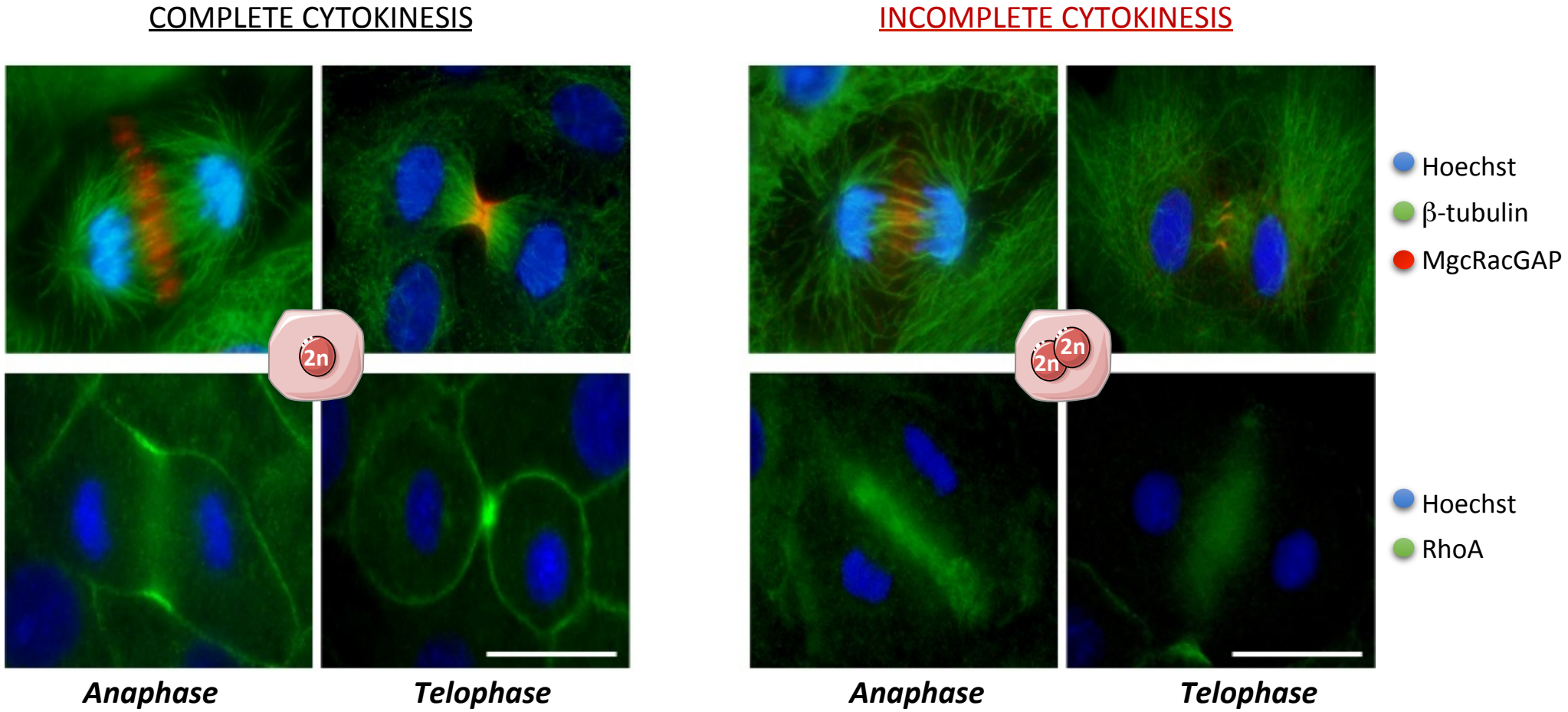

COMPLETE CYTOKINESIS
INCOMPLETE CYTOKINESIS
2n
2n
2n
Hoechst
β-tubulin
MgcRacGAP
Hoechst
RhoA
Anaphase
Telophase
Anaphase
Telophase

Figure 4:

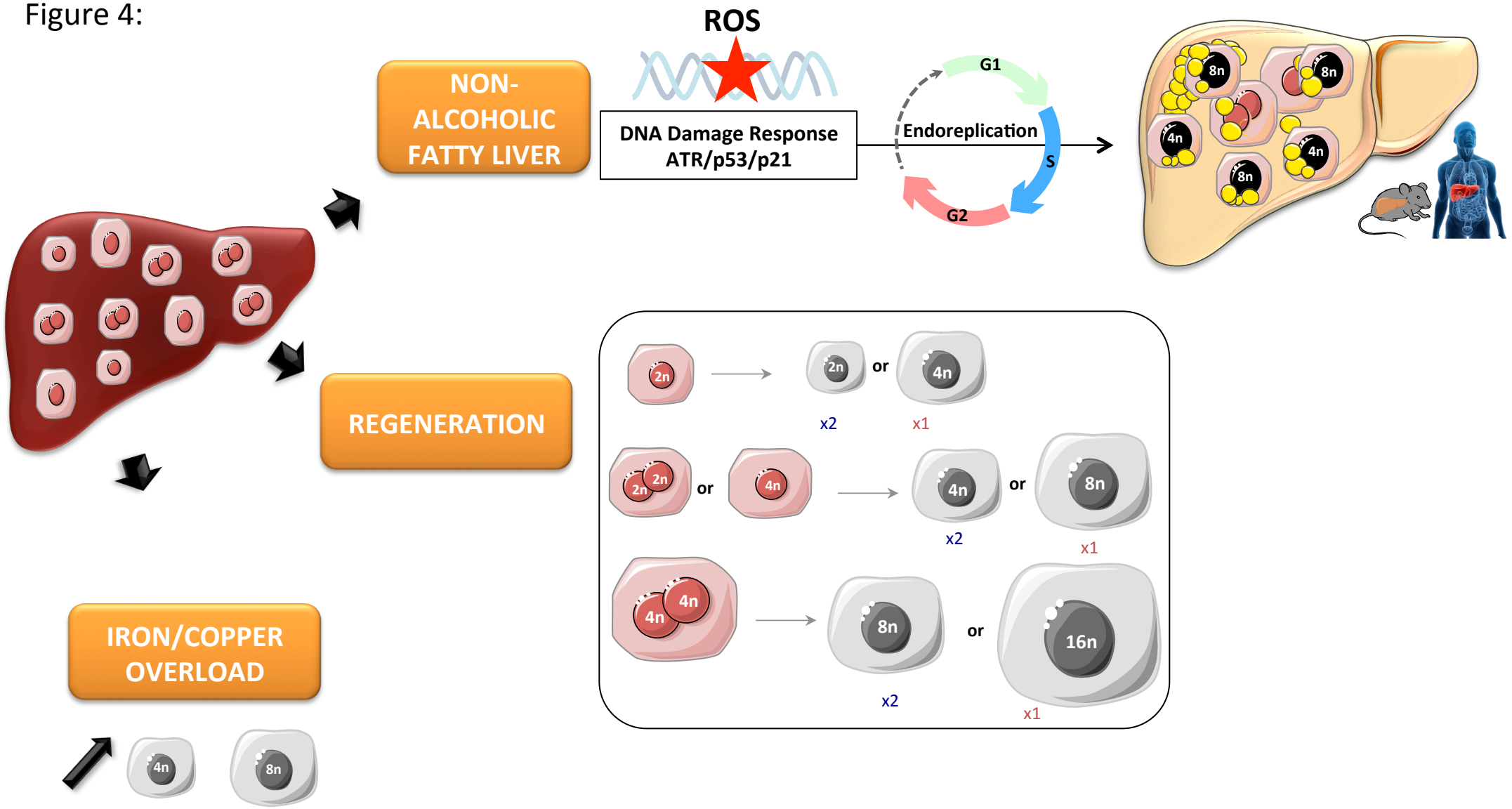
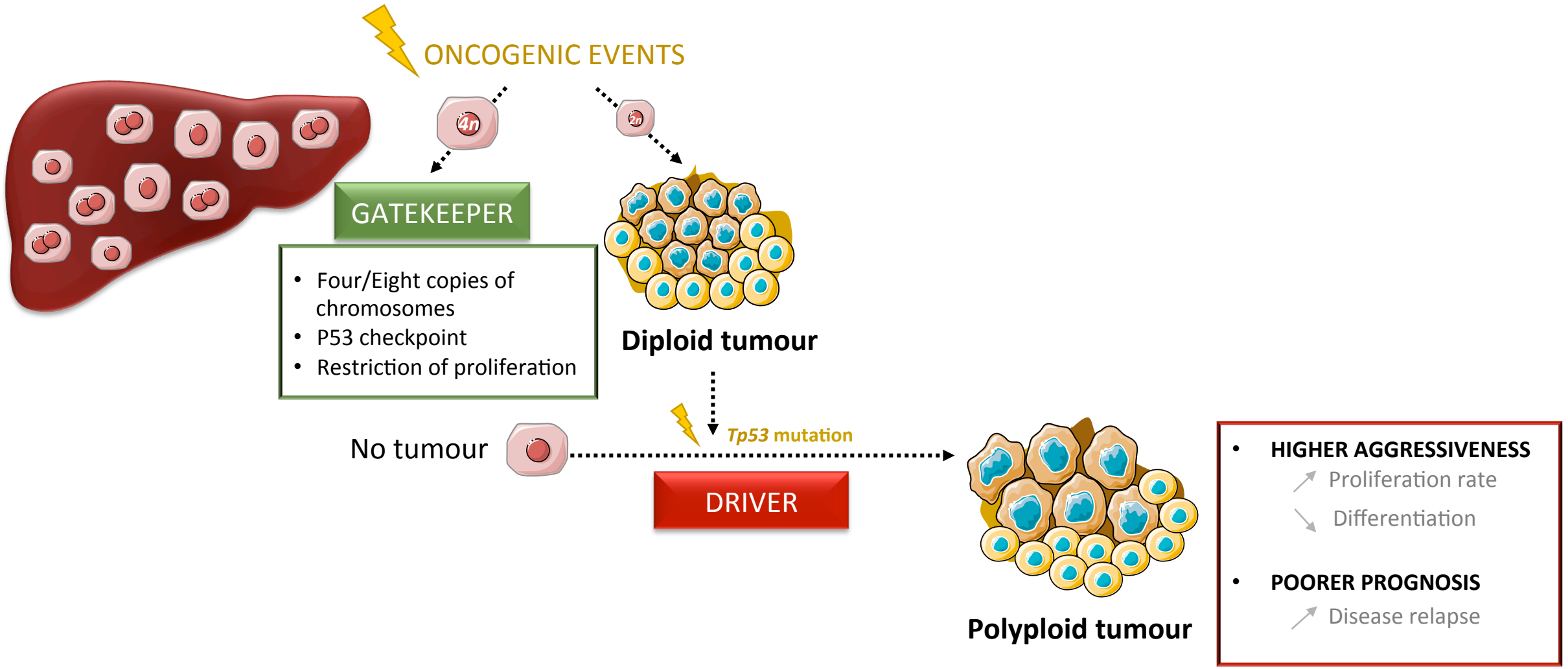

ROS
NON-ALCOHOLIC FATTY LIVER
DNA Damage Response ATR/p53/p21
G1
Endoreplication
S
G2
8n
4n
REGENERATION
2n
or
x2
x1
IRON/COPPER OVERLOAD
16n

Figure 5:


ONCOGENIC EVENTS
4n
2n
GATEKEEPER
• Four/Eight copies of chromosomes
• P53 checkpoint
• Restriction of proliferation
Diploid tumour
No tumour
Tp53 mutation
DRIVER
Polyploid tumour
• HIGHER AGGRESSIVENESS
Proliferation rate
Differentiation
• POORER PROGNOSIS
Disease relapse