disease. So far, most of our knowledge of NF-κB signaling in liver pathophysiology is derived from studies in which NF-κB activation or activity is inhibited. The authors used the opposite approach trying to activate NF-κB by removing CYLD. Surprisingly, only hepatocytes in close vicinity to the portal triad suffer spontaneous apoptosis but only after postnatal day 10, arguing against a cell autonomous promotion of cell death in the absence of CYLD. If this is the case, what triggers the periportal cell death from postnatal day 10 onward? Pertinently, the authors point to commensal bacteria in the gut. During embryogenesis and shortly after birth, the gut is sterile and thus, the portal circulation to the liver is free of bacteria or microbial products. However, after the first postnatal days, the microflora start colonizing the gut, and an influx of microbial components, such as lipopolysaccharide (LPS) reaches the liver. At this point, if the hepatocytes are devoid of CYLD, an important attenuator of NF-κB signaling, they misinterpret the amount of LPS and other TLR agonists to which they are exposed. Invariably, this would lead to programmed cell death in hepatocytes that overreact to TLR agonists. Of note, CYLD is expressed in a gradient, being higher in the periportal region and lower close to the central vein, supporting an important role for CYLD in signal fine-tuning in the area where TLR agonist concentration can fluctuate the most. New investigations using germ-free *Cyld*$^{\Delta hep}$ mice would provide an important test of this hypothesis. After this initial trigger, the CYLD-deficient liver starts on a self-destruction spiral. The initial death of periportal hepatocytes triggers an inflammatory response mediated by Kupffer cells. These cells produce TNF and other death cytokines that act on hyper-responsive hepatocytes, causing more cell death and more inflammation, eventually enhancing compensatory proliferation. Interestingly, Dapito et al. (2012) recently reported that HCC promotion is affected by intestinal microbiota through TLR4 signaling in the liver. Considering that in several human liver diseases there is an increase in intestinal permeability and consequently an increase of microbial components in the portal circulation, the studies of Nikolaou et al. (2012) and Dapito et al. (2012) establish a new paradigm in liver disease in which intestinal microbiota can determine hepatocyte cellular survival and death. Thus, the intestinal barrier and commensal microflora further influence the NF-κB-JNK interplay that is already known to play a central role in the control of liver pathophysiology.

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"Two" Much of a Good Thing: Telomere Damage-Induced Genome Doubling Drives Tumorigenesis

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Data from human tumors and mouse models suggest that tetraploidy, one example of polyploidy, can promote tumorigenesis. In this issue of *Cancer Cell*, Davoli and De Lange make important connections between tetraploidy, tumorigenesis, and telomere crisis—a common event during the development of human cancers.

Organism-level polyploidy-increased complete sets of chromosomes are very frequent in plants and are often found in insects, fish, and amphibians. Genome doublings are less common in metazoans, but at least two tetraploidization events are thought to have occurred during the evolution of vertebrates. Although still debated, it has been proposed that during

evolution, the extra chromosomes coming from a genome doubling could provide fodder for evolutionary experimentation, with the extra chromosomes being mutated, broken, and rearranged while viability is maintained by the set of intact chromosomes (Otto, 2007). This is an appealing idea that could be relevant to tumorigenesis. Indeed, tetraploid cells lacking the p53 tumor suppressor, generated by cytokinesis failure, are tumorigenic, and the resulting tumors display markedly altered genomes (Fujiwara et al., 2005).

The role of polyploidy in certain cell lineages during development is interesting but even more poorly understood. Polyploidy occurs in many metabolically active cells—hepatocytes, cardiomyocytes, trophoblast giant cells, and retinal pigment epithelial cells, to name a few (Davoli and de Lange, 2011). The correlation between polyploidy and metabolic activity has led to the speculation that polyploidy might confer a metabolic benefit, but the nature of this postulated benefit is entirely mysterious. Perhaps these poorly understood benefits drive polyploidization during the development of some cancers.

Although in special circumstances polyploidy may confer some benefits, it is not always a good deal. Newly-generated tetraploid cells, in organisms as diverse as yeasts and humans, exhibit mitotic defects and very high rates of whole chromosome aneuploidy (Davoli and de Lange, 2011). In mammals, most if not all of the chromosomal instability that accompanies tetraploidy is due to the presence of extra centrosomes. For example, it has been possible to isolate tetraploid cells in vitro that have spontaneously lost their extra centrosomes, and these cells exhibit normal genetic stability (Ganem et al., 2009). Indeed, most polyploid cells in mammalian tissues do not divide or only rarely divide, presumably because of the genetic instability that results from cell division of polyploid cells. When dividing cells do accidentally become tetraploid, a stress response is triggered that, with variable penetrance, can activates p53 and limit cell proliferation (Margolis et al., 2003).

Even though most mammalian cells are not programmed to become tetraploid, tetraploidy can occur as a consequence of a variety of pathological events. Errors in DNA replication or mitosis can lead to chromatin trapped in the cleavage furrow, eventually causing cytokinesis failure and tetraploidization. After a prolonged mitotic arrest, due to the activation of the spindle checkpoint, cells can undergo "mitotic slippage" and become tetraploid. Tetraploidization may also occur by cell fusion, for example, induced by viruses (Davoli and de Lange, 2011).

More recently, a surprising new mechanism to induce tetraploidy was uncovered: the endoreduplication of chromosomes (the absence of mitosis between two rounds of DNA replication) in the face of a persistent DNA damage signal, either from unprotected telomeres or from DNA damaging agents (Davoli et al., 2010). Deprotected telomeres are of particular interest because telomeres have been found to shorten during the early stages of many epithelial cancers. When p53-deficient cells suffer persistent DNA damage, DNA damage response kinases are activated. Among other substrates, these kinases phosphorylate and inactivate the Cdc25 phosphatases, thus blocking CyclinB/Cdk1 activity and preventing mitotic entry. However, Davoli and de Lange (2012), in this issue of *Cancer Cell*, found unexpectedly that the cell cycle does not halt, but rather flips between the G2 and the G1 phase without nuclear envelope breakdown or mitosis. During these endocycles, the origins of DNA replication are first licensed in G1, cells then enter S phase and complete DNA synthesis, but after a period of time in G2, geminin, a key inhibitor of DNA re-replication, is eventually degraded, allowing the entire cycle noted above to be repeated if the cells lack p53 (Davoli et al., 2010).

The new paper from Davoli and de Lange (2012) goes more deeply into the underlying mechanism and directly establishes that these events can promote tumorigenesis. In their previous work, Davoli and de Lange had induced telomere crisis in mouse cells somewhat artificially by genetic ablation of *Pot1*, which encodes a key protein required for telomere protection (Davoli et al., 2010). Here, they show that endoreduplication occurs in cells undergoing a more "physiological" telomere crisis, using late passage telomerase-negative cells. In a variety of human cell types, tetraploidization occurs either by endoreduplication, as described above, or by cytokinesis failure, with different proportions of these events observed in different cell types (Figure 1). The development of large numbers of tetraploid cells not only requires loss of p53, but also loss of the Rb tumor suppressor. Loss of Rb prevents a G1 arrest that would otherwise be imposed by activation of the CDK inhibitor p16. Finally, after transiently inactivating telomere protection in p53-deficient mouse embryo fibroblasts, diploid and tetraplopid cells were separated by FACS-sorting and tested for their transforming potential. Satisfyingly, the tetraploid cells exhibited significantly greater tumorigenic activity than the diploid cells carried through the same isolation procedure. Analysis of tumor karyotypes showed that the tumor genomes had evolved a subtetraploid chromosome content. The generation of unstable tetraploid cells, followed by evolution to a subtetraploid genome may occur in human tumors, as was recently inferred from genomic analysis of metastases from a renal carcinoma (Gerlinger et al., 2012).

Altogether, these findings show that telomere attrition is a double whammy. It is already known that critical telomere shortening can lead to chromosome breaks and rearrangements via breakage-fusion-bridge cycles. The new findings reveal that tetraploidy adds further fuel to the fire. Importantly, both sources of instability are potentially reversible. After telomere crisis, breakage-fusion-bridge cycles are short-circuited by reexpression of telomerase. Likewise, the transformed tetraploid cells generated by Davoli and de Lange (2012) evolved to presumably more stable subtetraploid genomes (Figure 1). Transient genetic instability may be beneficial to a developing cancer cell; it increases the chances of obtaining transforming mutations but obviates the need to pay the fitness cost of ongoing instability.

This work raises a host of interesting biological and mechanistic questions. First: if nascent tumor cells go through a genome doubling, at what point in tumor development does that occur? Recent work by Carter et al. (2012) took a computational approach to analyze DNA copy number data from a huge number of tumors. This study confirms that genome doublings and near-tetraploid genomes are remarkably common.

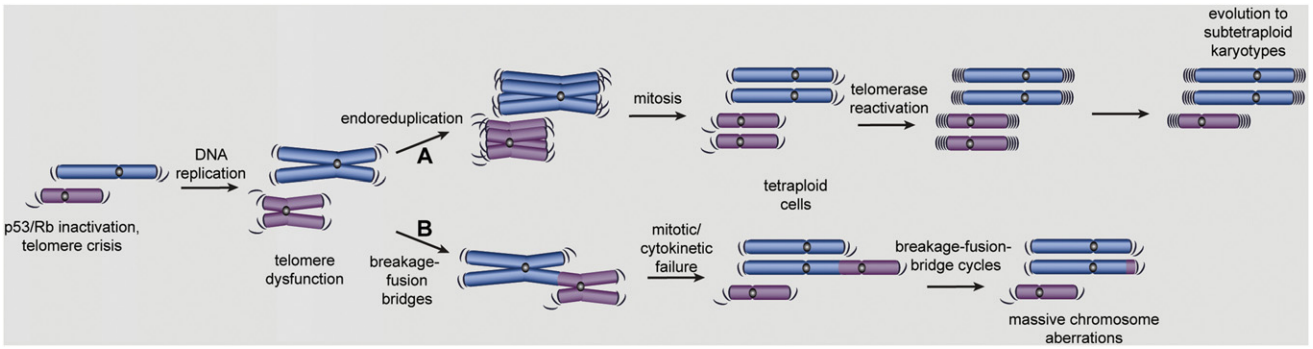

Figure 1. Telomere Dysfunction Promotes Tetraploidy

(A) Endoreduplication after prolonged DNA damage of p53 deficient cells. Telomere dysfunction, or other persistent DNA damage, generates a DNA damage response that arrests p53 deficient cells in the G2 phase of the cell cycle. After a prolonged arrest, diploid (2N) cells degrade the DNA replication inhibitor geminin, license DNA replication origins, and start a new round of DNA synthesis without entering mitosis. The resultant tetraploid cells survive and proliferate if telomerase is re-expressed. This tetraploid clone can promote tumorigenesis and often evolves to a subtetraploid karyotype.

(B) Tetraploidization after cytokinesis failure. In the setting of deprotected telomeres, cells can develop telomere end-to-end fusions. This generates dicentric chromosomes and initiates chromosome breakage-fusion-bridge cycles. The dicentric chromosomes generated by this mechanism can result in chromatin trapped at the cleavage furrow and subsequent failure of cytokinesis. Both mechanisms of tetraploidization result in centrosome amplification and additional chromosomal instability.

However, their data suggest that genome doublings often occur relatively late, after the acquisition of other chromosome aneuploidies. This is perhaps not that surprising given the need for p53 loss to enable the proliferation of polyploid cells. Also, polyploidy need not always be integral to tumor development; in some circumstances, it may be consequence rather than cause. Second: what is the mechanism by which polyploidy accelerates oncogenesis? Recent work has revealed that errors in mitotis can cause DNA damage and thus potentially produce cancer-causing mutations (Crasta et al., 2012; Janssen et al., 2011). Given that centrosome amplification is instrumental in driving the instability of tetraploid cells (Ganem et al., 2009), a parsimonious hypothesis is that genome doublings may drive tumor development in the old-fashioned way—by generating mutations that activate oncogenes and inactivate tumor suppressors.

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