of hnRNP-K to specific genomic loci? Is there functional significance to the remarkable proximity (~15 kb) of lincRNA-p21 to the *p21* gene? If these loci are regulated interdependently, they may act as a key molecular switch between life and death. Finally, given the importance of lincRNA-p21 to p53-dependent cell death, is lincRNA-p21 mutated in cancer? Answers to each of these questions will certainly enrich our understanding of the functional relationship between p53 and this powerful class of regulatory molecules, lincRNAs.

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Stem Cells and DNA Damage: Persist or Perish?

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Stem cells repopulate tissues after injury while also renewing themselves, but this makes them vulnerable to genotoxic damage. Mohrin et al. (2010) and Milyavsky et al. (2010) now show that mouse and human hematopoietic stem cells make opposing decisions about whether to die or to persist in response to DNA damage.

Stem cells have the immense responsibility of maintaining tissue and organism homeostasis over the lifetime of an individual. As such, stem cells are speculated to have evolutionary characteristics that offer protection against acute insults, allowing them to survive and to repopulate their tissues in the short term. However, they must also act as self-renewing guardians of the genome to ensure maximal integrity of the genomic code for future stem cells and their mature tissue progeny. The hematopoietic (blood) system is perhaps the best studied tissue in terms of its hierarchical development from a small number of long-term stem cells that are relatively quiescent, to progenitors that proliferate and differentiate, and then to mature blood cell lineages that are produced by the billion each day. Hematopoietic stem cells are thought to be resistant to injury including DNA damage, which may be related to their specific gene expression programs, epigenetic factors, or exogenous protection by the stem cell "niche." Two new reports in *Cell Stem Cell* from the laboratories of Emmanuelle Passegué (Mohrin et al., 2010) and John Dick (Milyavsky et al., 2010) further our understanding of how hematopoietic stem cells respond to radiation-induced DNA damage.

So how do quiescent stem cells handle genotoxic insults? Mohrin et al. (2010) found that murine hematopoietic stem and progenitor cells (HSPCs)—defined as bone marrow cells expressing the markers: lineage$^-$/c-Kit$^+$/Sca-1$^+$/Flk2$^-$—were more resistant to apoptosis induced by a specific dose of ionizing radiation than were more differentiated progenitor cells (Figure 1). The unique DNA damage response of mouse HSPCs involves the tumor suppressor protein p53 and is lost when stem cells are forced out of quiescence and into the cell cycle by treatment with chemotherapy or cytokines. Not only are quiescent HSPCs poised to resist apoptosis as evidenced by their antiapoptotic gene expression program, but they are also able to repair their DNA by nonhomologous end joining (NHEJ). Repair of DNA damage through homologous recombination (which has a lower error rate than NHEJ) requires that cells enter the cell cycle; thus, quiescent stem cells must rely on NHEJ as an alternative. The reliance of quiescent adult tissue stem cells on NHEJ for the repair of DNA damage may in fact be a general

phenomenon in mice, given the similar conclusions of a recent study using hair follicle stem cells as a model system (Sotiropoulou et al., 2010).

Unfortunately, Mohrin et al. (2010) also uncover a downside to short-term radioresistance and rapid DNA repair through the error-prone NHEJ pathway. Spectral karyotyping revealed gross chromosomal aberrations in irradiated mouse HSPCs, and some of the same abnormal cytogenetic findings persisted in the progeny of irradiated HSPCs transplanted into mouse recipients. Furthermore, despite their resistance to apoptosis immediately after injury, irradiated HSPCs were unable to contribute to long-term sustained hematopoiesis when serially transplanted into mouse recipients. Such events would be of obvious risk to a long-lived organism as serial exposure of stem cells to genotoxic agents could readily result in leukemia or aplasia.

Does the human hematopoietic system accept the same tradeoff between stem cell survival in the short term versus accumulation of deleterious mutations in the long term? In a companion study, Milyavsky et al. (2010) addressed this question in human umbilical cord blood cells. They observed an enhanced sensitivity to apoptosis induced by low-dose ionizing radiation in these cells compared to more differentiated cells. In contrast to the findings of Mohrin et al. in the mouse, these authors noted that human hematopoietic stem and early multipotent progenitor cells were poised for apoptosis in response to DNA damage. Survival and the clonogenic and reconstitutive capacity of the irradiated human HSPCs were rescued by blocking p53 expression or by overexpression of the antiapoptotic factor Bcl-2. However, irradiated human HSPCs lacking p53 were unable to sustain hematopoiesis and showed evidence of persistent DNA double-strand breaks when serially transplanted into recipient mice. Therefore, a short-term gain in survival could be achieved by human hematopoietic stem cells as found in the mouse, but the default setting for irradiated human HSPCs is an increase in p53 expression resulting in apoptosis.

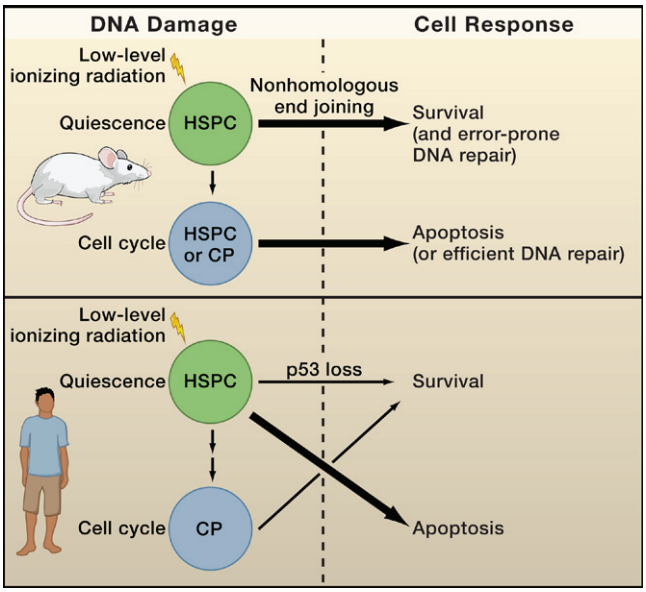

Figure 1. Hematopoietic Cell Responses to DNA Damage

(Top) Quiescent murine hematopoietic stem and progenitor cells (HSPCs) are poised to survive DNA damage induced by low-level ionizing radiation through a DNA repair process called nonhomologous end joining (NHEJ), which tends to be error prone (Mohrin et al., 2010). In contrast, mouse HSPCs and committed progenitors (CP) progressing through the cell cycle are more likely to undergo apoptosis or repair their DNA using higher-fidelity homologous recombination. Although the short-term consequence of HSPC survival is maintenance of tissue integrity in the face of injury, long-term consequences include genomic rearrangements that persist and HSPCs with a diminished functional capacity.

(Bottom) In contrast, compared to more committed progenitors, the default program for damaged human HSPCs is to undergo apoptosis. However, a decrease in p53 rescues human HSPCs from apoptosis immediately after low-level irradiation (Milyavsky et al., 2010). Despite interspecies differences in the short-term response to radiation, the long-term functional consequences of avoiding apoptosis for both mouse and human HSPCs include persistent DNA damage and decreased self-renewal capacity. The delicate balance between tissue survival and the DNA damage response therefore could predispose surviving HSPCs to future malignant transformation.

The differences between these two studies may have a technical basis: markers for stem and progenitor populations are more refined in the mouse than in the human so somewhat different stem and progenitor cell populations may have been analyzed. In addition, slightly different doses of radiation were used. In the in vivo experiments of Milyavsky et al., human HSPCs from umbilical cord blood were transplanted into the mouse bone marrow niche, which may have provided less efficient survival signals for human cells than for mouse cells. Also, human umbilical cord blood HSPCs have a different biology than the bone marrow HSPCs of mouse. However, it is tempting to see the different findings in the light of evolution, that is, as a reflection of the different challenges faced by mammals with different life spans and ages of reproductive maturity.

Recent elegant studies from Bondar and Medzhitov (2010) and Marusyk et al. (2010) demonstrate that competitive selection takes place within tissue stem cell populations. These authors found that irradiated p53-deficient HSPCs in the mouse have an initial survival advantage but that long-term fitness is balanced by complex interactions with neighboring HSPCs and the relative levels of p53 and DNA damage in stem cells and their neighbors. The studies from the Passegué and Dick labs indicate that hematopoietic cells within a tissue have adopted different means of handling DNA damage depending on their differentiation stage. That mouse and human stem cells may have acquired or undergone selection for distinct responses to ionizing radiation is a reasonable notion. However, it remains to be seen which specific molecular mechanisms that differ between stem cells and progenitors, or between stem cells of different species, lead to these distinctive traits. We now have a set of reagents with which to discover and understand how such important yet different tissue stem cell traits have evolved.

There are other practical implications of the Passegué and Dick reports. Secondary myelodysplasia and leukemia are believed to arise from DNA damage to HSPCs from the radiation or chemotherapy given to treat a primary malignancy. Mohrin et al. show intriguing evidence that NHEJ activity and chromosomal aberrations decrease when HSPCs are induced to enter the cell cycle prior to irradiation. Interestingly, a parallel evolving hypothesis in the study of cancer stem cells sug-

gests that activating leukemia stem cells from quiescence prior to chemotherapy may result in more efficient elimination of these cancer-repopulating cells (Saito et al., 2010). An unexpected benefit of such a prestimulation strategy may be that normal hematopoietic stem cells activated from quiescence would simultaneously be protected from accumulating long-term DNA damage. However, as shown by Milyavsky and colleagues, stem cell escape from acute damage, particularly if it involves a decrease in p53 activity, may lead to long-term deleterious effects on stem cell fitness and repopulating ability. The interplay between the response to acute injury and long-term fitness needs to be more fully understood and will require both laboratory models and the thoughtful correlative study of stem cells from patients receiving genotoxic chemotherapy. Understanding these events may point the way to methods for preserving short-term tissue reconstitution while maintaining long-term cell and genomic integrity.

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Mitochondrial Matrix Reloaded with RNA

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Although mitochondrial biogenesis requires the import of specific RNAs, the pathways and cellular machineries involved are only poorly understood. Wang et al. (2010) now find that polynucleotide phosphorylase in the intermembrane space of mammalian mitochondria facilitates import of several RNAs into the mitochondrial matrix.

Mitochondria, the power plants of the eukaryotic cell, are bound by two membranes and contain 1000–1500 different proteins and tens of RNAs. Most of the genes that encode mitochondrial proteins are found in the nuclear genome and thus are translated in the cytosol and then imported into mitochondria. The pathways and machineries required for protein import into mitochondria have been extensively studied and are highly conserved among fungi, plants, and mammals (Endo and Yamano 2009; Chacinska et al., 2009). The mitochondrial matrix also contains several kinds of noncoding RNAs that are also imported from the cytosol. However, in contrast to protein translocation, the mechanisms that mediate import of RNAs into mitochondria remain enigmatic (Salinas et al., 2008; Lithgow and Schneider, 2010). In this issue of *Cell*, Wang et al. (2010) shed light on this question, revealing that polynucleotide phosphorylase (PNPase) is a much sought after component of the RNA import apparatus in mammalian cells.

PNPases comprise an evolutionally conserved enzyme family (found in bacteria, plants, flies, and mammals but not in yeast) that has 3′→5′ exoribonuclease and RNA-polymerase activities (Chen et al., 2007). Although bacterial PNPases are cytosolic, eukaryotic PNPases are mainly localized in mitochondria or chloroplasts. Prior work has established how PNPases get to the intermembrane space (IMS). After crossing the mitochondrial outer membrane via the translocase of outer mitochondrial membrane 40 (TOM40) complex, the PNPase precursor engages with the translocase of the inner membrane 23 (TIM23) complex (Figure 1) (Chen et al., 2006; Rainey et al., 2006). After the PNPase presequence is removed by matrix processing peptidase (MPP), an AAA protease Yme1 in the inner membrane pulls PNPase into the IMS, where PNPase assembles into a trimeric complex (Figure 1).

Wang et al. now assess the function of mammalian PNPase by tissue-specific disruption of the *PNPase* gene in mouse hepatocytes. They find that mitochondria from hepatocytes deficient in PNPase display defects in oxidative phosphorylation (OXPHOS), the major ATP-generating metabolic pathway of respiration. This defect is shown to arise from the failure in the processing of polycistronic mitochondrial mRNAs encoding the