Stem Cell DNA Damage and Genome Mutation in the Context of Aging and Cancer Initiation

Lara Al Zouabi, Allison Bardin

Stem Cell DNA Damage and Genome Mutation in the Context of Aging and Cancer Initiation

Lara Al zouabi[1,2] and Allison J. Bardin*[1,2]

[1] Institut Curie, PSL Research University, CNRS UMR 3215, INSERM U934, Stem Cells and Tissue Homeostasis Group, Paris, France.
[2] Sorbonne Universités, UPMC Univ Paris 6, Paris, France.

Allison Bardin E-mail: allison.bardin@curie.fr
Tel: +33 1 56 24 65 62;

* Author for correspondence.

Abstract

Adult stem cells fuel tissue homeostasis and regeneration through their unique ability to self-renew and differentiate into specialized cells. Thus, their DNA provides instructions that impact the tissue as a whole. Since DNA is not an inert molecule, but rather dynamic, interacting with a myriad of chemical and physical factors, it encounters damage from both endogenous and exogenous sources. Damage to DNA introduces deviations from its normal intact structure and if left unrepaired, may result in a genetic mutation. In turn, mutant genomes of stem and progenitor cells are inherited in cells of the lineage, thus eroding the genetic information that maintains homeostasis of the somatic cell population. Errors arising in stem and progenitor cells will have a substantially larger impact on the tissue in which they reside than errors occurring in post-mitotic differentiated cells. Therefore, maintaining the integrity of genomic DNA within our stem cells is essential to protect the instructions necessary for rebuilding healthy tissues during homeostatic renewal. In this review, we will first discuss DNA damage arising in stem cells and cell- and tissue-intrinsic mechanisms that protect against harmful effects of this damage. Secondly, we will examine how erroneous DNA repair and persistent DNA damage in stem and progenitor cells impact stem cells and tissues in the context of cancer initiation and aging. Finally, we will discuss the utility of invertebrate and vertebrate model systems to address unanswered questions on the role that DNA damage and mutation may play in aging and pre-cancerous conditions.

Stem cells and tissue dynamics

Many adult metazoan tissues maintain long-term function through the ongoing elimination of terminally differentiated cells and the replacement of these cells by the newly divided progeny of cycling cells. An understanding of this process began almost 70 years ago through early lineage tracing studies of Charles Philippe Leblond using tritiated-thymidine injection in mice to reveal the turnover rates of labeled cells (Leblond and Walker 1956). These seminal studies initiated the stem cell theory of renewal and laid the foundation for modern labeling studies of cell turnover that confirmed age-associated mosaicism of adult tissues (Arrojo e Drigo et al. 2019; Spalding et al. 2013). Importantly, this work raised important conceptual questions regarding how stem cells may endure the process of aging.

Aging is associated with an alteration in stem cell functionality and kinetics of tissue renewal in many tissues such as blood, skin, muscle, and the brain (Kuhn et al. 1996; Morrison *et al.*, 1996; Conboy *et al.*, 2003; Nishimura et al. 2005). An imbalance in tissue dynamics due to deregulated self-renewal or cell turnover rates can compromise tissue function. Understanding how tissue dynamics are altered during aging or in pathological contexts is an important, yet highly complex question. At a molecular level, these changes may be induced by genetic, epigenetic or metabolic alteration. Potential causes may include changes in stem-cell-intrinsic factors, alteration of niche properties, or modification of systemic signals. In this review, we will focus on stem cell-intrinsic alteration through DNA damage and genetic mutation. For recent reviews on the impact of epigenetic and metabolic changes on aging stem cells, please see (Brunet and Rando 2017; Booth and Brunet 2016; Chandel et al. 2016).

DNA damage and how it leads to mutation

All cells, including stem cells, are faced with the challenges of protecting their DNA from erosion. DNA damage is a deviation from the normal DNA structure with the introduction of damaged sites in the base-pairing or backbone structure. Multiple exogenous agents such as UV light, ionizing radiation and chemical mutagens, such as hydrocarbons present in tobacco smoke, can damage DNA. In addition, endogenous factors such as reactive oxygen species, telomere erosion, and replication errors can also be a source of damage. DNA replication, for example, is an opportune time for error, as the replication fork can slow down, or collapse, due to topological challenges including limiting nucleotides, repetitive sequences, non-B-form DNA, and collisions with transcription machinery.

It has been estimated that tens of thousands of lesions are experienced by a mammalian cell per day, with single-strand lesions making up the majority of this number (Lindahl and Nyberg, 1972; Lindahl, 1974; Lindahl and Barnes, 2000). Damage involving a single-strand can be accurately repaired using the other strand as a template. Small base lesions that do not significantly change the DNA helix structure are repaired by base-excision repair (Lindahl 1974). As for misincorporated bases, they are corrected by the mismatch repair pathway (Lahue *et al*. 1989). On the other hand, lesions involving bulky adducts, and dimers are repaired by nucleotide-excision repair (Sancar 1993) whereas interstrand crosslinks require the Fanconi anemia pathway (Zhang and Walter 2014). Additionally, DNA double-strand breaks also arise in the cell, often through replication fork collapse. This type of damage is particularly dangerous because more error-prone repair mechanisms are used that can lead to the loss of genetic information, contributing to genome instability. DNA double-strand break repair is primarily orchestrated by two pathways: if the cell has gone through S phase, duplicating its chromosomes, providing a template for the repair of the damaged chromosome, homologous recombination (HR) is used. If, on the other hand, the cell is in G1, the more erroneous non-homologous end joining (NHEJ) pathway is used, which involves the ligation of the broken ends and the introduction of small deletions as a result.

Despite the existence of these strategies to safeguard the integrity of DNA, glitches in the system arise frequently leading to sequence variants, structural variants, or aneuploidy. Sequence variants include indels and point mutations, arising, for example, through deamination of 5-methylcytosine in CpG nucleotides in vertebrates, resulting in a C—>T substitution (Razin and Riggs 1980). Structural variants involve more large-scale changes to the DNA sequence and therefore are more likely to alter gene function. These include amplifications, deletions, and translocations, which can be caused by recombination and replication-based mechanisms, erroneous DNA double-stand break repair, or be a result of transposable element mobility (Carvalho and Lupski 2016; Bourque et al. 2018). Most genes are haplosufficient (Huang et al. 2010) and therefore inactivation of one copy may not impair cell function. However, problems may arise when one allele in the genome is already inactive in the germline and the second allele is inactivated somatically, leading to loss of heterozygosity (LOH). LOH can be driven by the aforementioned mutagenetic processes as well as recombination with the homologous chromosome, also known as “mitotic recombination”, which upon cell division, leads to segregation of two mutant alleles into one daughter cell. Thus, DNA damage in stem or progenitor cells

can alter the genome in numerous ways and potentially radically disrupt tissue function over the course of aging.

Some of the first evidence suggesting a potential causal link between cellular DNA damage and organismal aging came from the realization that inactivation of DNA repair genes such as Fanconi anaemia and Werner syndrome in humans lead to early aging or “progeroid” syndromes (Carrero et al. 2016; Moskalev et al. 2013). Due to reduced ability to evade DNA damage via repair, DNA lesions persist, and somatic mutations accumulate. Patients with these syndromes exhibit accelerated aging and present symptoms of loss of proper tissue renewal such as skin atrophy, loss and graying of hair, and higher susceptibility to cancer development. While these studies suggest sufficiency of DNA damage to drive early aging phenotypes, they do not provide evidence that endogenous levels of DNA damage or mutation can impact aging. How DNA damage and genome mutation may impair stem cell function will be further discussed below. We will first examine mechanisms that can mitigate the effects of DNA damage in stem cells.

Mechanisms protecting the stem cell and tissue from the effects of DNA damage

While coping with DNA damage is important for all cells, it is particularly vital for adult stem cells that renew tissues throughout adult life. What are the ways in which stem cells and tissues avoid the negative impact of DNA damage and mutation? Here, we will discuss a number of important protection mechanisms acting at the stem cell and tissue level.

Protecting the stem cell: DNA damage responses and repair

Evidence suggests that at least some stem cells employ distinct mechanisms from their downstream differentiated or more committed progenitor cells to prevent the accrual of genetic lesions, which can be detrimental to homeostasis of the tissue. DNA damage is managed via the DNA damage response (DDR), which is an evolutionarily conserved signaling pathway where sensors, mediators and effectors orchestrate DNA repair or by the elimination of the damaged cell by apoptosis or by exiting the cell cycle. Interestingly, adult mouse hematopoietic stem cells (HSCs) and hair follicle stem cells (HFSCs) of the bulge were found to have increased radioresistance with minimal apoptotic response and accelerated DNA repair compared to their more differentiated progeny (Mohrin et al. 2011; Sotiropoulou et al. 2010; Beerman et al. 2014). Aged HSCs are even more resistant than young HSCs to DNA-damage induced apoptosis (Gutierrez-Martinez et al. 2018). This likely helps prevent depletion of the stem cell pool but could be at the cost of accumulating mutations.

Stem cells also differ in a tissue-dependent manner in terms of strategies used that help limit passing mutations to progeny, with some favoring robust repair (Fig. 1A), others apoptosis (Fig. 1B), or terminal differentiation (Fig. 1C). The small intestine for instance is sensitive to apoptosis driven by DNA damage, whilst stem cells of the colon are resistant to apoptosis (Potten and Grant 1998; Merritt et al. 1995). Intrinsic differences in cell cycle properties could explain why stem cells differ widely in their DNA repair mechanisms between tissues. As shown in the hematopoietic system, when a DSB arises in a quiescent cell, DNA repair is mediated by the efficient NHEJ mechanism, which acts quickly and does not need the presence of a homologous DNA for repair, but is error-prone (Mohrin et al. 2011).

Proliferating HSCs on the other hand use high-fidelity HR to repair DSBs but have an increased likelihood of accumulating damage during S-G2/M (Mohrin et al. 2011). Alternatively, another strategy of protecting the tissue from propagating a mutation is employed by melanocyte stem cells that differentiate upon DNA damage (Nishimura et al. 2005; Inomata *et al.*, 2009). Similarly, during the aging process, it is thought that the HFSC are gradually lost due to differentiation upon repeated DNA damage acquisition during hair follicle cycles (Fig. 1C) (Matsumura et al. 2016).

Caught between balancing the need to maintain tissue function and the need to block the propagation of mutations, stem cells have evolved diverse modes to cope with DNA damage and repair, often sacrificing immediate survival of a given stem cell for the expense of long-term maintenance of genome-integrity in the tissue. Further studies are important to better understand the sensitivity and resistance of adult stem cells to damage, the repair mechanisms employed, and age-related changes in this process.

Protecting the stem cell: A quiescent state

One way to limit DNA damage is simply to avoid undergoing cell division, which would restrict replicative and chromosome segregation errors (Fig. 1C). Indeed, many populations of adult stem cells including hematopoietic, muscle, and neural stem cells are primarily in a non-proliferating quiescent state of G0 (van Velthoven and Rando 2019; Cho et al. 2019). Evidence suggests that quiescence serves a protective role in these contexts as these populations of stem cells become depleted or "exhausted" when driven into the cell cycle upon transplantation, due to stress, or upon genetic manipulation (Chen *et al.*, 2000; Harrison, 1978; Gan *et al.*, 2010; Sacco *et al.*, 2010; Schaniel *et al.*, 2011; Staber *et al.*, 2013; Cavallucci et al. 2016; Yue *et al.*, 2016; Baumgartner *et al.*, 2018; Singh *et al.*, 2018; Kamminga et al. 2006). Stem cell exhaustion in these contexts may be due to loss of niche signals. Alternatively, these studies raise the possibility that increased DNA damage or an increased mutational burden upon loss of quiescence may lead to stem cell functional decline during aging (Sharpless and DePinho 2007). Consistent with this notion, when mouse HSCs were forced repeatedly out of quiescence they acquired DNA damage and became depleted (Walter et al. 2015). Nevertheless, the extent to which stem cell exhaustion is related to increased DNA damage or acquisition of mutations is not entirely clear and may differ depending on stem cell type. Additional potential links between DNA damage and stem cell senescence will be discussed later in the review.

Protecting the tissue: Competition between cells and lineages

In addition to stem-cell intrinsic mechanisms of protection mentioned above, tissue-level protection also helps to ensure the survival of the fittest lineage. This may be especially important for stem cells such as the Crypt Basal (Lgr5+) intestinal stem cells (ISCs) and those of the skin epidermis, that actively divide. One such mechanism is neutral competition in which ISCs undergo dynamic stem cell replacement shown, both in the mouse (Lopez-Garcia et al. 2010; Snippert et al. 2010) and the *Drosophila* intestine (De Navascués et al. 2012), which likely helps to prevent their loss.

Aside from neutral competition, biased cell competition also occurs between cells. Initially described in *Drosophila*, biased cell competition is a phenomenon whereby differences in cellular fitness

allow selection of “winner” cells, while weeding out less-fit “loser” cells (Fig. 2A) (Morata and Ripoll 1975). A large body of work in *Drosophila* has revealed that this process plays an important role in shaping adult tissues and has elucidated many molecular mechanisms underpinning this process. For a review of the topic see (Levayer 2019). Competition between stem cells also occurs and can result in greater niche occupancy of a given genotype with selective advantage or greater production of progeny (Nystul and Spradling 2007; Jin et al. 2008; Issigonis et al. 2009; Kolahgar et al. 2015; Zhang and Kalderon 2001; Amoyel and Bach 2014).

An important question, however, is what types of fitness differences are being sensed during cell competition? Could stem cells with DNA damage or with less fit mutant genomes be selected against? Interestingly, mouse HSCs that have been treated with low dose ionizing radiation (IR) are less competitive than non-irradiated HSCs in a manner that is dependent on Trp53 levels and last for weeks (Fig. 2B) (Bondar and Medzhitov 2010). This implies that a memory of the irradiation stress was kept, which is proposed to be linked to a Trp53-dependent long-term mark acting as a cellular memory for DNA damage. A recent study of the mouse skin demonstrated that stem cell lineages compete based on levels of the hemidesmosome component, COL17A1 (Fig. 2C) (Liu et al. 2019). Interestingly, like HSCs, exposure of epidermal stem cells to IR triggers a long-lasting memory of genomic stress, resulting in the proteolytic degradation of COL17A1. How this memory is achieved and whether it also relies on p53, unrepaired DNA damage, or could be linked to genomic mutations or epigenetic mechanisms, is not clear. Another recent study of the mouse skin epidermis showed, as previously demonstrated in *Drosophila* (de la Cova, 2004; Moreno and Basler 2004), that stem cell lineages with higher levels of *Mycn*, a bHLH transcription factor, become winners (Fig. 2A, C). It is not currently clear whether, like COL17A1, *Mycn* might respond to altered genomic stress and how these two mechanisms might overlap. Interestingly, mechanisms that may be akin to cell competition can also expunge aberrant tissues with altered tissue architecture, such as those expressing oncogenic *Hras* GTPase, as demonstrated in mouse hair follicle using live imaging (Brown et al. 2017). This is very reminiscent of early work in the fly showing elimination of tumorigenic cells via cell extrusion (Brumby and Richardson 2003; Vaughen and Igaki 2016). Thus, cell and lineage competition are mechanisms that can help to maintain integrity of adult tissues and are likely one means of eliminating cells with harmful DNA damage or mutant genotypes.

When protection mechanisms fail: acquisition of mutation

Despite the numerous mechanisms in place to protect stem cells from harmful effects of DNA damage, studies over the past 10 years revealed the extent to which genomic mutations arise in adult stem cells. Here we will present data demonstrating that genetic changes occurring in stem or progenitor cells contribute to tissue mosaicism. We will also highlight some of the recent literature from humans that has demonstrated that somatic genetic mosaicism is not a rare, pathological event, but a phenomenon present in many of our healthy adult tissues.

Evidence of surprising diversity in somatic genomes

Finding and studying somatic mutations in subsets of cells within a tissue is extremely challenging. While recent advances in genomic sequencing are beginning to unveil the extent to which somatic variation arises, classic genetic studies using visible marker phenotypes provided the first evidence of genetic mosaicism. Studies by Curt Stern using *Drosophila* first demonstrated spontaneous loss of heterozygosity (LOH) during development due to mitotic recombination between homologous chromosomes (Stern 1936). Mitotic recombination is an important mechanism of LOH in cancer and other genetic disorders (Jonkman et al. 1997; Choate et al. 2010), though not yet well understood in healthy tissues. Somatic variation due to mobilization of transposable elements was later studied in maize by Barbara McClintock (1950). Evidence from reporter mice and DNA sequencing-based approaches suggest that LINE1 element mobility contributes to genetic mosaicism in the nervous system (Coufal et al. 2009; Erwin et al. 2016; Upton et al. 2015; Muotri et al. 2005) and estimate a de novo LINE1 element insertion frequency of 0.2 events per neuron in humans (Evrony et al. 2012). See (Faulkner and Garcia-Perez, 2017) for a more extensive review of this literature. How somatic mobilization of transposable elements impacts adult tissues, is only beginning to be understood. Additional mutagenic processes also shape somatic mosaicism. Sequencing clonally expanded human adult stem cells using organoids has demonstrated that around 40 de novo point mutations are acquired per year in liver, colon, and small intestine (Blokzijl et al. 2016); 13 de novo point mutations mutations per year in muscle stem cells (Franco et al. 2018); and about 200-400 total point mutations impact neural precursors (Lodato et al. 2018). One prominent mutational signature found in both human and mouse precursors is C-to-T transitions at CpG dinucleotides, thought to be due to deamination of 5-methylcytosine to thymine (Behjati et al. 2014; Blokzijl et al. 2016; Lodato et al. 2015). In addition, larger-scale gene deletion and rearrangements were detected using SNP array methodology, with around 14% of human colon crypts bearing a large-scale deletion or LOH event (Hsieh et al. 2013), which has been also documented in other tissues (O'Huallachain et al. 2012). Whole-genome sequencing of colon also recently confirm SNP and copy number changes in healthy tissue (Lee-Six et al. 2019). Aneuploidy and copy number variation in the brain and other tissues have similarly been reported, though frequencies vary depending on the detection technique (Rehen et al. 2002; Cai et al. 2014; O'Huallachain et al. 2012, Knouse *et al.* 2014). Thus, it is now abundantly clear that human tissues have high degrees of genetic mosaicism. It is, therefore, critical to perform functional studies to understand the full impact of mosaicism on young, aged, healthy and diseased adult tissues.

Clonal expansion in blood and solid tissues

Mosaic patches of adult tissue, or "clones", can result from a long-lived stem or progenitor cell acquiring a mutation driving positive selection due to increased fitness, or from neutral drift of an alteration with no impact on fitness (Snippert et al. 2010; Traulsen et al. 2013). Evidence for age-dependent clonal expansion of mutant stem cell lineages in the blood dates back to the 90s where probes for the inactive X-chromosome were used and detected its skewing during aging (Fey et al. 1994; Busque et al. 1990). More recently, the study of "healthy" control blood using sequencing-based

approaches led to surprising evidence for clonal expansion of lineages having somatic mutation in the genes *TET2*, *DNMT3a,* and *ASLX1* during adult aging (Busque et al. 2012; Jacobs et al. 2012; Laurie et al. 2012; Holstege et al. 2014; Welch et al. 2012). We will discuss further the physiological implications of blood clonality below but for an extensive review on clonal haematopoiesis see (Jaiswal and Ebert 2019).

Mounting evidence similarly indicates that solid tissues also have a high degree of genetic mosaicism with mutant progenitor cells giving rise to expanding mutant lineages under positive selection. In the 90s, it was recognized with PCR and through whole-mount tissue staining that sun-exposed normal human skin acquires clones of mutant *TP53* (Nakazawa et al. 1993; Jonason A S et al. 1996). In recent years, these finding were greatly extended using targeted deep sequencing of 74 cancer driver genes on biopsies of normal sun-exposed eyelid epidermis and normal esophagus tissue. Frequent mutation of genes was found, including in *NOTCH1* and *TP53,* that expand clonally and accumulate with age (Martincorena *et al.*, 2015, 2018; Yokoyama *et al.*, 2019). Additional recent evidence for large clonal expansions across numerous tissues including breast and lung has been demonstrated with mutational analysis of RNAseq data (Yizhak et al. 2019). Furthermore, other tissues show clear examples of somatic mutation-driven clonal expansion. In humans, megaencephaly syndromes leading to a clonal overgrowth of part of the brain arise through activating mutations of the AKT/PI3K pathway that can be due to somatic mutations arising in neural precursor cells (Lee et al. 2012; Rivière et al. 2012; Lodato et al. 2018; Poduri et al. 2012). Interestingly, somatic mutations activiating PI3K have also been found to lead to Proteus syndrome, with patients having overgrowth of fibrous and adipose tissues (Lindhurst et al. 2012). Thus, positive selection of mutant lineages is prevalent in human tissues. The implications on cancer initiation of somatic mutations in driving early lineage expansion and selection will be further discussed below.

DNA damage and somatic mutation in adult tissues: roles in cancer initiation and aging

What is the impact of these mutations on tissues? Clearly cancer initiation is one detrimental consequence, but not all mutations lead to cancer. Here we will highlight the functional implications of somatic genetic mosaicism.

Somatic mutations and cancer initiation

For over a hundred years, it has been recognized that cancer cells are distinct from normal ones due to the presence of aberrant genomes (Boveri 1914).Therefore, recent revelations that normal tissues harbor extensive mutations, raise important questions about the relationship between apparently healthy tissue and cancer: do mutations that provide positive selection in a tissue actually promote the eventual acquisition of additional genetic mutations leading to cancer as described in a classical multistep carcinogenesis model? Alternatively, in some instances, might these be two distinct selection processes with cancer requiring a divergent path from one that optimizes growth within an otherwise healthy tissue? As we previously discussed, multiple modes of cell and lineage competition

actively shape the nature of selection within a tissue and, in theory, could respond differently to expanding mutant lineages versus precancerous clones.

Evidence from clonal hematopoiesis supports a multistep process where a first mutation in healthy tissue precedes additional mutation (Fig. 3), increasing cancer risk. Indeed, longitudinal studies of patients with clonal hematopoiesis detected by SNP arrays support a strong increased risk of developing not only hematological cancer (Laurie et al. 2012; Jacobs et al. 2012; Welch et al. 2012; Genovese et al. 2014; Jaiswal et al. 2014; Coombs et al. 2017), but also lung and kidney cancers (Jacobs et al. 2012). Exome sequencing revealed that known tumor suppressor genes of myeloid cancers such as *TET2, DNMT3A* and *ASXL1*, were mutated in apparently healthy blood (Busque et al. 2012; Genovese et al. 2014; Jaiswal et al. 2014; McKerrell et al. 2015; Coombs et al. 2017). Thus, the acquisition of these mutations in healthy blood is thought to represent the earlier phase in the development of leukemogenesis and suggests a period of latency that precedes it. Therefore, an understanding of how processes such as stem cell competition for niche occupancy may influence the switch from a premalignant state to a malignant one is important (Fig. 3B,D).

Recent studies in the skin and esophagus support the idea of healthy tissue acquiring premalignant drivers, but also suggest the intriguing possibility that healthy tissues may have distinct selective pressures than those in cancer. Targeted deep sequencing of normal oesophageal epithelium from young and old donors revealed that the number of detectable mutations and the sizes of mutant clones increased with donor age (Martincorena, *et al.*, 2018). *NOTCH1* and *TP53*, canonical drivers of Esophageal squamous cell carcinoma (ESCC), were found to be under selection in normal tissue (Martincorena *et al.*, 2018b; Yokoyama *et al.*, 2019). Thus, the presence of clonal expansions in the normal epithelium suggests that these clones have a premalignant capacity and their persistence can lead to cancer initiation (Fig. 3B,D). These data strongly support the concept of “field cancerization” (Slaughter and Southwick 1953), previously proposed to predispose the esophagus to development of subsequent multiple tumors via initial precancerous drivers such as p53 (Tian et al. 1998). Nevertheless, an intriguing finding is that mutations in *NOTCH1* and *PPM1D* are much more prevalent in normal skin than in cancer (Martincorena *et al.*, 2018b; Yokoyama *et al.*, 2019). This suggests that different fitness of certain mutations exist in “normal” tissue versus cancer, complicating the notion of a linear multistep mutation accumulation process. Future studies will be necessary to understand these fitness differences and potentially capitalize on them for clinical benefit.

An impact of mutations and DNA damage on aging?

Aside from initiating and driving cancer evolution, what impact do somatic mutations have on aging? Here we will discuss some of the potential detrimental consequences of mutation on tissues.

Studies from clonal hematopoiesis have demonstrated a collapse of clonal diversity with very few stem cells contributing to the aging blood (Fig. 3C). This results from “winner” HSC clones expanding and, in an apparent zero-sum game, “loser” HSCs failing to contribute to blood. This was strikingly demonstrated from sequencing the blood of a hematologically asymptomatic supercentenarian (aged 115 years old) revealing that approximately 65% of her healthy blood compartment was dominated by the progeny of two hematopoietic stem cell (HSC) clones (Holstege et

al. 2014). Extending on earlier work that we discussed above (Busque et al. 2012; Jacobs et al. 2012; Laurie et al. 2012; Welch et al. 2012), a study using whole-genome sequencing from the peripheral blood of ~11,000 Icelanders of different ages found that a striking 50% of patients older than 85 had clonal hematopoiesis (Zink et al. 2017). Thus, abundant evidence indicates that mutations arise in HSCs (or in very upstream precursor cells) during aging and lead to selection of mutant lineages, however, the functional impact of collapse of clonal diversity is still not fully understood. One feature of the aging hematopoietic system in humans and mouse is a bias towards myeloid lineages (Sudo et al. 2000; Ganuza et al. 2019; Yamamoto et al. 2017). While unlikely to explain all of the myeloid bias of HSCs that occurs during aging, *TET2* deletion is sufficient in mouse to lead to a myeloid disorder (Li et al. 2011) and is strongly associated with myeloid dysplasia in humans (Buscarlet et al. 2018). Thus, a failure to maintain the repertoire of differentiated cell types present in youth can arise from a loss of clonal diversity. Interestingly, a reduction in the clonality of mouse muscle stem cells upon repeated injury was found (Tierney et al. 2018). While the role of mutation or DNA damage was not evoked in this study, it is feasible that increased replication stress might indeed drive some stem cell lineages to contribute less to the tissue, possibly explaining the observed collapse in clonality in the muscle.

Hypercompetitive lineages may render other lineages “losers”, but deleterious mutations may also create “loser” lineages cell-autonomously through suboptimal growth, stem cell functional decline, or loss from the tissue of the stem cell or lineage. Is there evidence for this? Quantifying deleterious mutations is a difficult task as these mutations will be either lost or only be present in a few cells. As a work-around, techniques from evolutionary biology have been applied to look at negative selection of point mutations within somatic tissues. By considering the normalized ratio of non-synonymous to synonymous mutations, one can deduce the amount of detrimental mutations which had been lost. Strikingly, no evidence of negative selection was found in human tissues or in numerous types of cancer (Martincorena et al. 2017; Franco et al. 2018), arguing that the arising point mutations were not detrimental to the survival of the cell in which they arose. It is not yet clear how other types of mutational processes may create burdens on the cell or be selected against. For example, it is more likely that large-scale deletions or mitotic recombination-based LOH, both affecting hundreds to thousands of genes, would reduce cellular fitness. Similarly, de novo transposition events may also impair cellular function through transcriptional deregulation. The extent to which this occurs or might trigger cell death or cell selection mechanisms at the tissue level, is not yet known.

Contributions of persistent DNA damage to stem cell decline

A large body of literature, including work on HSCs, NSCs, and muscle stem cells, has explored the effects of persistent, induced DNA damage and has demonstrated the sufficiency of DNA damage to drive early aging phenotypes. We will not extensively review this literature here but refer the reader to some excellent reviews of the subject (Williams and Schumacher 2017; Niedernhofer et al. 2018). While much of this work is not exclusively on stem cells, collectively these studies demonstrate that unrepaired DNA damage can perturb general cellular function in a number of ways including: 1. Leading to cell cycle arrest, 2. Driving apoptosis or cellular senescence, 3. Physically disrupting transcription (Garinis et al. 2009), 4. Causing large transcriptomic changes including growth signaling and metabolic

pathways (Edifizi et al. 2017), 5. Altering chromatin organization through relocalization of factors to DNA damage sites (Oberdoerffer et al. 2008).

This work raises the question of whether endogenous levels of DNA damage can impact aging and if so, by which mechanisms. Several studies demonstrate a link between increased cellular senescence and stem cell functional decline during aging. An increase in the expression of the senescence-associated cyclin-dependent kinase inhibitor, $p16^{INK4a}$, was observed during aging in HSCs, NSCs, pancreatic islet cells, and muscle stem cells, accompanied by a decreased functionality of these stem cell populations during aging that was ameliorated in $p16^{INK4a}$-/- mice (Janzen et al. 2006; Krishnamurthy et al. 2006; Molofsky et al. 2006; Sousa-Victor et al. 2014). While these data support the notion of $p16^{INK4a}$-dependent effects on stem cells, it should be noted that $p16^{INK4a}$ need not be activated through endogenous DNA damage, but could be linked to one of the DNA damage-independent modes of *p16* activation, such as changes to chromatin (Martin and Beach 2014). Consistent with this, a loss of silencing via BMI1 repression of $p16^{INK4a}$ was shown to underlie muscle stem cell senescence (Sousa-Victor et al. 2014).

Ongoing DNA damage may also result from alteration in replication kinetics. Interestingly, in mouse adult HSCs, diminished expression of MCM4 and MCM6 during aging resulted in delayed replication kinetics in aged HSCs causing replication stress. Induced replication stress in HSCs resulted in preferential killing of old HSCs, therefore providing a mechanism for functional decline of cycling HSCs during aging (Flach et al. 2014) and a likely explanation for previous observations in human and mouse HSCs, of increased marks of DNA damage during aging (Rossi et al. 2007; Rübe et al. 2011).

Despite these findings suggesting that DNA damage may impair stem cell activity, the effects on adult stem cells of persistent DNA damage versus genome mutation or DNA damage signaling, must be further teased apart. In addition, determining how different types of endogenous DNA damage or mutagenic processes impact adult stem cells will be important. Finally, future studies are needed to define tissue-specific differences in endogenous DNA damage and their effects on stem cells and niche signals.

Towards an understanding of DNA damage and mutation in adult tissues

With the recent influx of DNA sequencing of healthy human tissues with age, our views regarding the genomes of somatic cells have been radically challenged. While these studies provide a descriptive snapshot of evolving somatic genomes, the use of genetically amenable model systems will further improve our understanding of molecular causes and tissue-wide consequences of endogenous DNA damage and somatic mutations in adult stem cells.

Model systems to quantify and study spontaneous mutation in tissues

Early model system studies investigating spontaneous mutation accumulation with age *in vivo* did so exploiting a transgenic mouse and *Drosophila* lines with an integrated *LacZ* reporter gene allowing quantification of mutation at this locus (Dolle et al. 2000; Garcia et al. 2010; Busuttil et al. 2007; Giese et al. 2002; Dolle 2002). An age-dependent increase in spontaneous mutation and an intriguing

tissue bias of *LacZ* mutations was found: while mostly point mutations were found in the small intestine, large genome rearrangements were found in the heart (Fig. 4A) (Dolle et al. 2000). Though these studies only focus on a single, artificial transgene, they provided an important foundation to begin to study spontaneous mutation *in vivo*.

Other studies using *in vivo* lineage tracing of mutant stem cells in the mammalian intestine allowed for the better understanding of stem cell dynamics and the fixation of mutations with age. Kozar and colleagues used mice containing a dinucleotide repeat tract within a reporter gene to mark if strand slippage happens during DNA replication that consequently resulted in an in-frame reporter gene, marking the cell. Intestinal crypts that are wholly populated by these marked mutations increased with age (Fig. 4B) (Kozar et al. 2013). Similarly, in the human colonic epithelium, mutant stem cell dynamics were revealed by marking known spontaneous mutations that continuously label the ISCs. Interestingly, the authors found much slower kinetics of crypt clonality likely due to slower stem cell turnover in humans compared to mouse (Nicholson et al. 2018).

Our lab has recently developed a powerful model system to investigate spontaneously arising mutations in adult intestinal stem cells in *Drosophila* (Siudeja et al. 2015). We observed that during aging, spontaneously arising intestinal neoplasia develop in around ~12% of adult males over a rapid period of 6 weeks of adult life. Through application of whole-genome sequencing, we could demonstrate that these arise largely due to structural variants deleting regions of the *Notch* gene. As *Notch* is X-linked and present in a single copy in males, loss of one copy is sufficient to fully inactivate *Notch* and block proper stem cell differentiation, thereby resulting in the accumulation of large clonal masses of stem cells (Fig. 4C). In addition, we uncovered a second means of genome alteration through loss of heterozygosity likely through mitotic recombination (Siudeja and Bardin 2017; Siudeja et al. 2015). These data suggest that spontaneous mutation occurs frequently in *Drosophila* adult intestinal stem cells, making them a useful model to decipher underlying causes and consequences of stem cell somatic mutation on adult tissues. Important advantages of this model include the rapid acquisition of mutations over 6 weeks of aging, the application of whole-genome sequencing, abundant genetic tools, and the ability to alter environmental conditions.

Alternative model systems: diverse evolutionary strategies of somatic genome stability

In addition to fly and mouse models, other models are providing important advances in our understanding of effects of somatic DNA damage and mutation on adult stem cells and tissues. Active work in *C. elegans* has led to insight into systemic effects of somatic DNA damage (Mueller et al. 2015; Williams and Schumacher 2017). Further investigation in alternative invertebrate models such as planaria, hydra, and non-traditional vertebrate models such as the naked-mole rat may provide surprising solutions to how organisms cope with DNA damage or somatic mutations. Hydra and planaria, for example, have stem cells with an unlimited capacity for self-renewal and do not show signs of aging (Boehm et al. 2013) and the naked-mole rat is a long-lived vertebrate that is cancer resistant (MacRae *et al.*, 2015; Petruseva et al. 2017). Probing into mechanisms in these models may yield unanticipated new insight into potential ways to mitigate the negative effects of mutation.

Concluding remarks

In this review, we described how alterations to the DNA of stem cells can disrupt their efficient self-renewal and differentiation, consequently changing the status quo of different tissues, eventually impacting aging and cancer initiation. We highlighted some cell- and tissue-specific mechanisms by which stem cells protect themselves from damage and mutations. Despite these protection mechanisms, damage and acquisition of mutation occur. Ironically, somatic mutation and errors in the DNA repair process are capitalized on in the soma in some instances such as the generation of antibody diversity in vertebrates, reviewed in (Li et al. 2004) or in programmed genome rearrangement occurring in lamprey, actively eliminating potentially harmful germline genes from the soma (Smith et al. 2018; Wang and Davis 2014).

Over recent years, advances in the technology to detect mutations and rare events in asymptomatic healthy tissues have revealed the sobering fact that our tissues are peppered with mutations. Healthy tissues are actually mosaics of cell lineages derived from mutant stem and precursor cells. Future studies will better define the forces of selection in healthy tissues and how these relate to cancer. An additional challenge will be to unveil the functional impact of accumulating mutations, linking genotype to diseases and aging phenotypes.

Fundamental questions remain regarding how DNA damage and somatic mutation of stem cells can be manipulated to slow down aging and delay or evade cancer initiation. How can genomic damage be prevented from accumulating in stem cells? Might mechanisms of cell competition be harnessed to replace potentially harmful mutant cells with therapeutic cells? Can tissue extrinsic factors such as changes in the environment be manipulated to control clonal expansions? Indeed, these questions remain open today and will be active areas of research benefitting from studying diverse genetic model systems.

Acknowledgements

We thank B. Boumard, K. Siudeja and P-A Defossez for critical reading of the manuscript. A.B. is a Director of Research (DR2) at the CNRS (Centre National de la Recherche Scientifique). L.A. is supported by funding from the IC3i PhD program and Fondation pour la Recherche Medicale. Our research is supported by grants from Schlumberger Foundation of Education and Research (FSER, prize 2017), the Fondation pour la Recherche Médicale DEQ 20160334928, and funding from the program "Investissements d'Avenir" launched by the French Government and implemented by ANR SoMuSeq-STEM CES13-0012 (A.B).

Figures and Legends

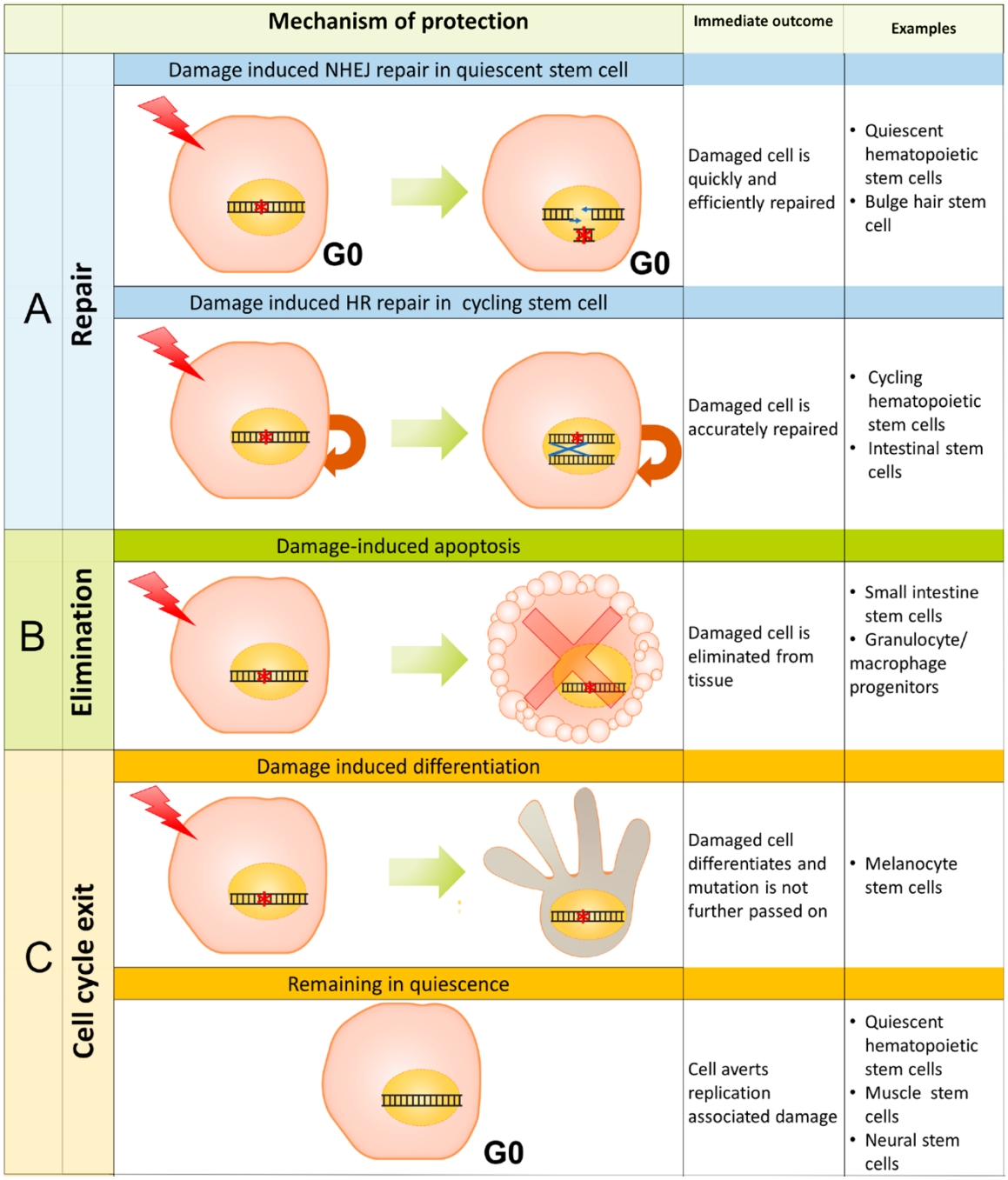

Figure 1. Mechanisms of stem cell protection from DNA damage by repair, elimination or cell cycle exit. (A) Repair: Depending on the cell cycle status of the cell, the cell undergoes repair by either non-homologous end-joining (NHEJ); (top panel) or homologous recombination (HR); (bottom panel). NHEJ is the quick and efficient mechanism employed by quiescent stem cells when they are faced with damage. It involves the ligation of the broken ends and often results in the introduction of small

deletions, but can also lead to translocation and genome rearrangements. HR is employed if the cell is cycling and goes through S phase,duplicating its chromosomes, providing a template for the repair of the damaged chromosome. This is usually more accurate repair than NHEJ, though erroneous choice of the homologous chromosome, rather than the sister, can lead to LOH. (B) Elimination: by apoptosis. Some cells undergo apoptosis rather than repair. If this mechanism is preferentially employed in the stem cell, there is a higher chance of stem cell depletion. (C) Cell cycle exit: by differentiation (top panel) upon DNA damage, or remaining in a state of quiescence.

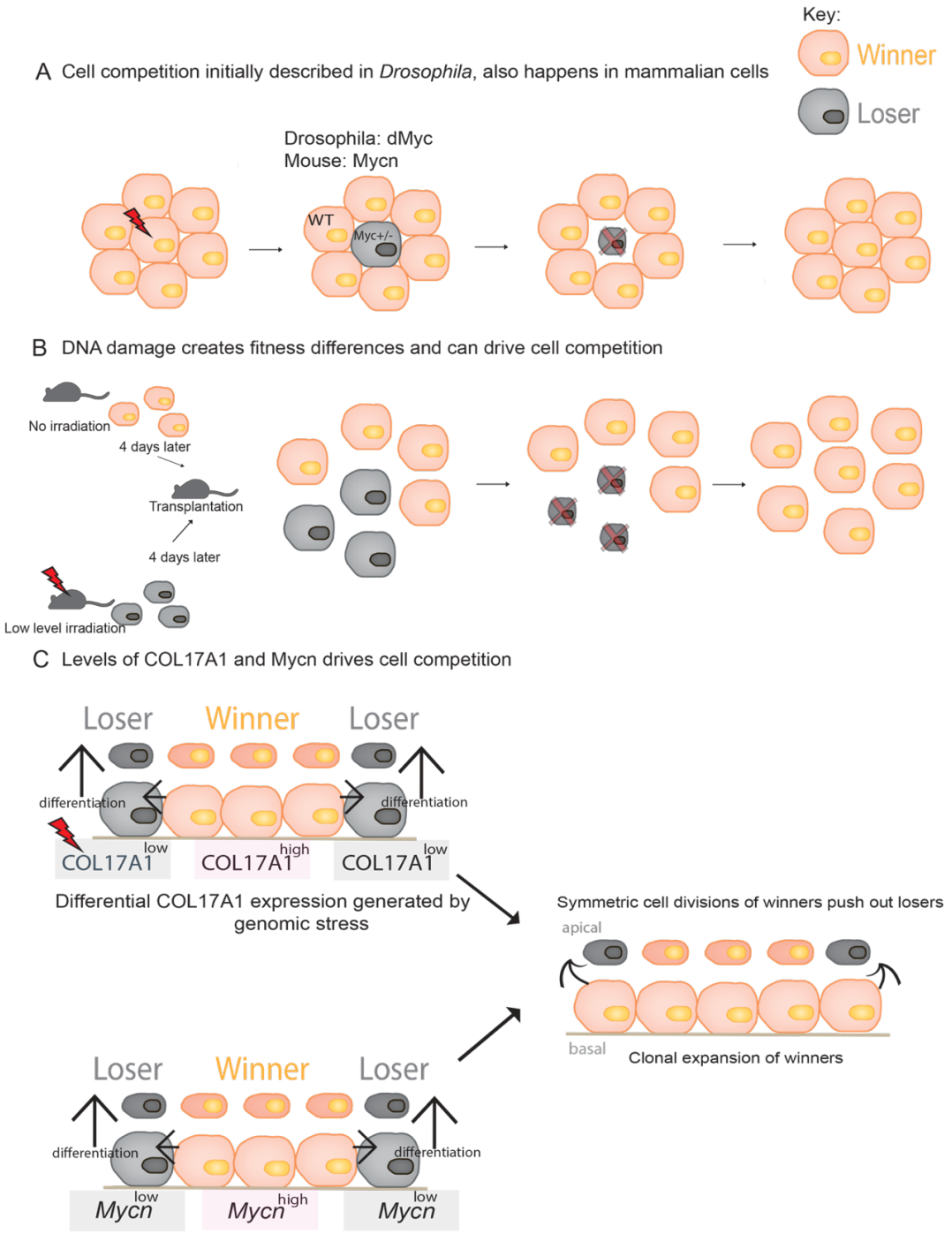

Figure 2. Cell competition selects for "winner" cells and weeds out less-fit "loser" cells. (A) Differential Myc levels drive cell competition in both *Drosophila* (progenitors in the disc) and mammalian cells (mouse developing epidermal cells and adult epidermis). This figure illustrates that Myc mutants are "losers" and are outcompeted by adjacent WT cells. Extra levels of Myc also renders cells "winners" compared to wild-type cells (not shown). (B) DNA damage creates differences in fitness and this can drive cell competition. Irradiation of mouse hematopoietic stem and progenitor cells creates DNA damage which results in increased Trp53 levels. When these cells are transplanted into mice with non-irradiated cells (4 days after the irradiation), the irradiated

cells are outcompeted by the non-irradiated cells that have lower Trp53 levels and a higher expression of more competitive signalling molecules. Thus, DNA damage via irradiation creates a long-lasting “loser” cell status by inducing p53-mediated apoptosis or cell cycle arrest. (C) Stem cell lineages with higher levels of COL17A1 and *Mycn* become “winners” in mouse skin epidermis. Genomic stress leads to the proteolytic degradation of COL17A1 and thus results in differential levels of COL17A1 expressed in the epidermis. Cells with higher levels of COL17A1 outcompete the cells expressing lower levels via symmetric cell division and the elimination of the losers. The higher expression of COL17A1 maintains a healthy skin phenotype, whereas COL17A1 deficiency causes skin

atrophy, fragility, dyspigmentation and alopecia. Similarly, skin lineages with higher levels of Mycn outcompete the cells expressing lower levels of *Mycn*, but it remains unclear whether genomic stress is what drives differential *Mycn* expression.

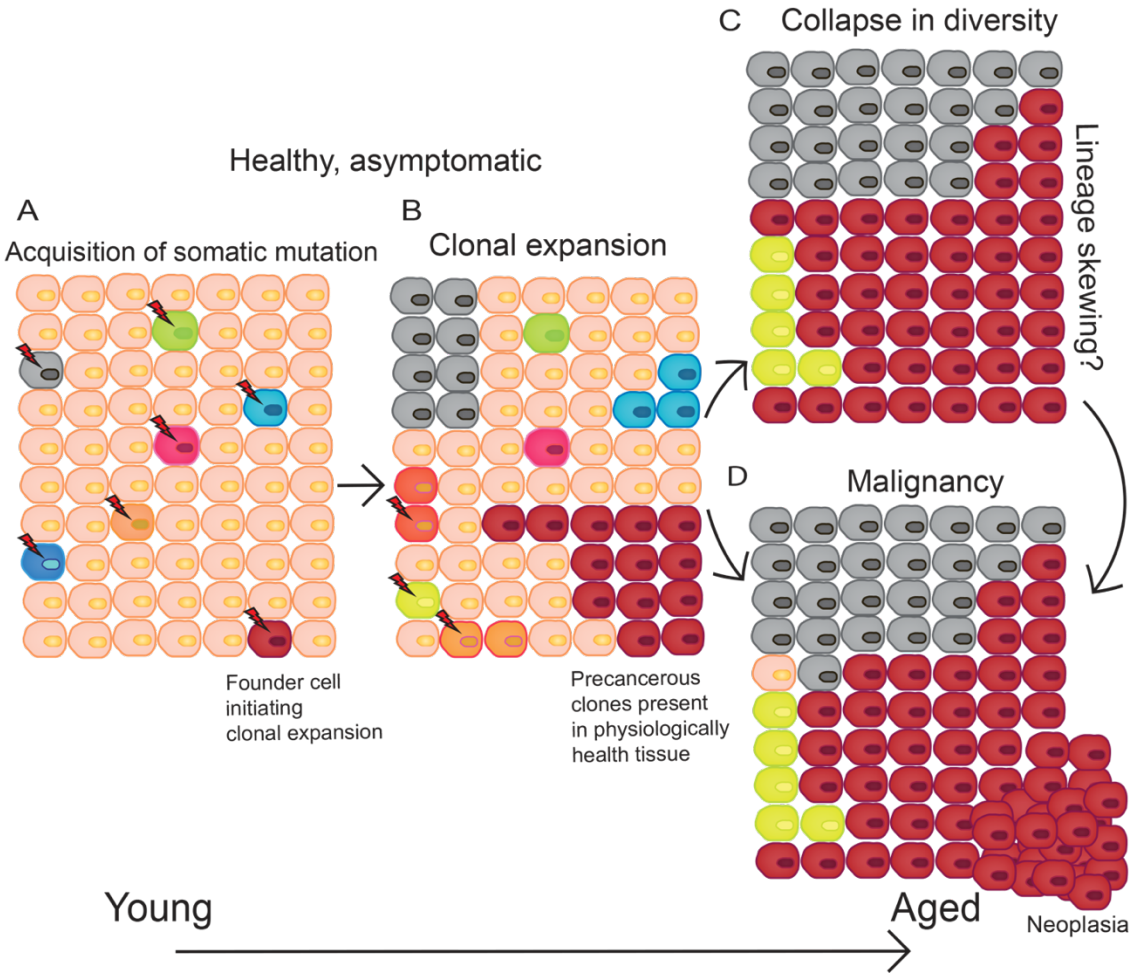

Figure 3. Somatic mosaicism with age. (A) Mutations arise in stem cells of young tissues. (B) Age-dependent clonal expansion of mutant stem cells via positive selection or neutral drift give rise to mosaic patches. These may have a premalignant capacity and their persistence can lead to cancer initiation. (C) Clonal expansion can lead to the age-related collapse in clonal diversity with very few stem cells contributing to the aging tissue. The functional impact of collapse of clonal diversity is still not fully understood but it can impact age-associated lineage skewing, in some cases. (D) Clonal expansion of cancer driver genes can lead to cancer initiation.

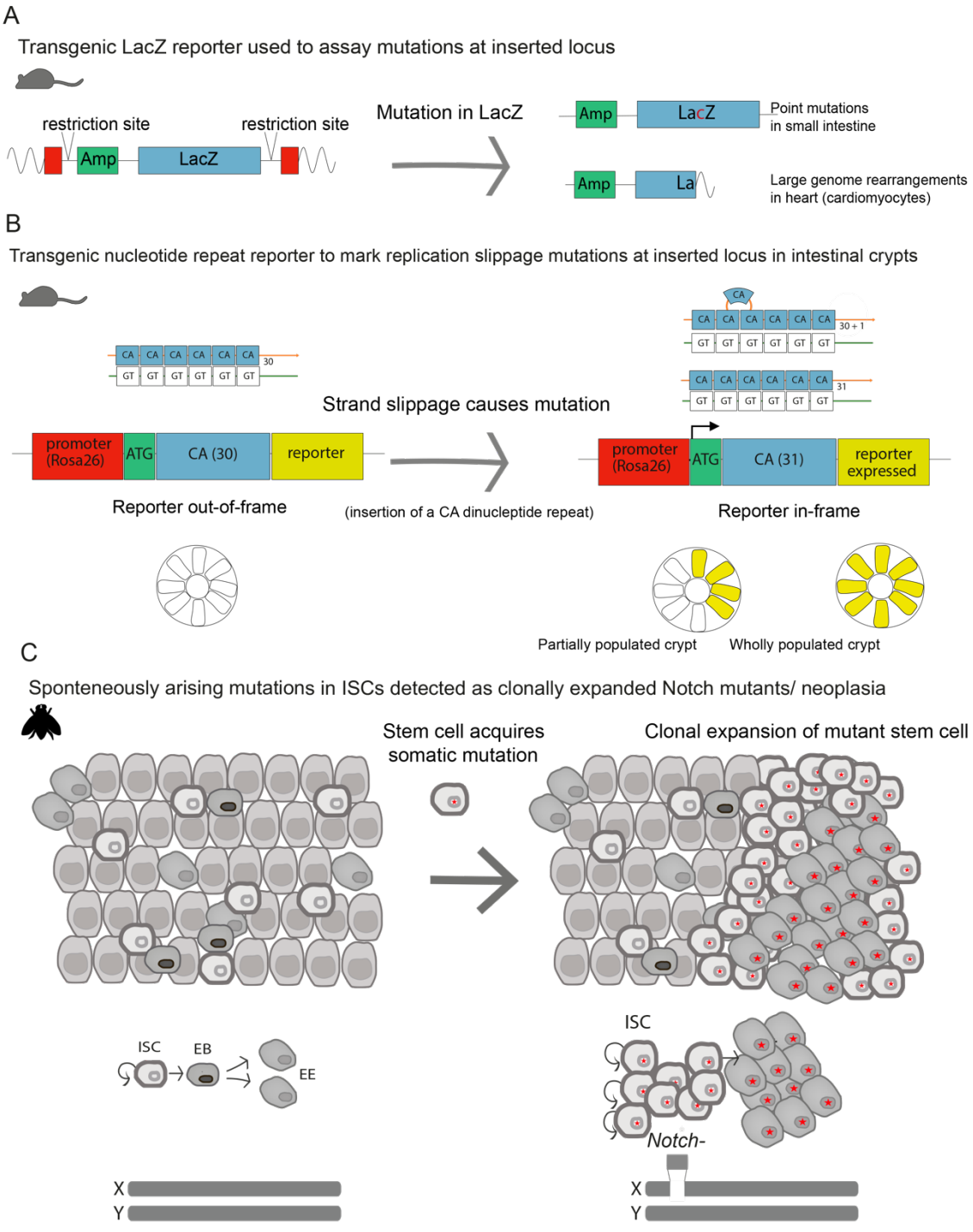

Figure 4. The use of model systems to assay spontaneous mutations in adult tissues. (A) A LacZ reporter to assay spontaneous mutations (Dolle et al. 2000). In this assay, genomic DNA extracted from young/old hearts and small intestines of transgenic mice containing the LacZ reporter is assessed for spontaneous mutation. LacZ encoding plasmids are recovered in bacteria tested for intact LacZ activity. A significant age related increase in point mutations was detected in small intestines whereas a significant age related increase in genomic rearrangements was detected in hearts. (B) A transgenic nucleotide repeat reporter assay to mark spontaneous mutations in mouse

intestinal crypts (Kozar et al. 2013). In this system, spontaneous slippage of the cassette during replication allows for expression of the reporter. Wholly populated crypts indicative of the fixation of mutations were shown to increase in an age-dependent manner. (C) Spontaneously arising somatic *Notch* mutations in *Drosophila* intestinal stem cells (ISCs) can be detected as clonally expanded *Notch* mutants/ neoplasia in aged flies (Siudeja et al. 2015).Inactivation of *Notch* leads to neoplasia with an accumulation of ISCs and EEs. Whole genome sequencing of aged male neoplasia revealed that *Notch* is inactivated via deletions or structural rearrangements.

References

Amoyel M, Bach EA. 2014. Cell competition: how to eliminate your neighbours. *Development* **141**: 988–1000.

Arrojo e Drigo R, Lev-Ram V, Tyagi S, Ramachandra R, Deerinck T, Bushong E, Phan S, Orphan V, Lechene C, Ellisman MH, et al. 2019. Age Mosaicism across Multiple Scales in Adult Tissues. *Cell Metab*.

Baumgartner C, Toifl S, Farlik M, Halbritter F, Scheicher R, Fischer I, Sexl V, Bock C, Baccarini M. 2018. An ERK-Dependent Feedback Mechanism Prevents Hematopoietic Stem Cell Exhaustion. *Cell Stem Cell* **22**: 879-892.e6.

Beerman I, Seita J, Inlay MA, Weissman IL, Rossi DJ. 2014. Quiescent hematopoietic stem cells accumulate DNA damage during aging that is repaired upon entry into cell cycle. *Cell Stem Cell* **15**: 37–50.

Behjati S, Huch M, Roerink S, Gundem G, van de Wetering M, Robinson B, van Boxtel R, Blokker J, Cuppen E, Campbell P, et al. 2014. Genome sequencing of normal cells reveals developmental lineages and mutational processes. *Nature* **513**: 422–425.

Blokzijl F, De Ligt J, Jager M, Sasselli V, Roerink S, Sasaki N, Huch M, Boymans S, Kuijk E, Prins P, et al. 2016. Tissue-specific mutation accumulation in human adult stem cells during life. *Nature* **538**: 260–264.

Boehm A, Khalturin K, Erxleben FA, Hemmrich G, Ulrich C, Lopez-quintero JA, Oberg H, Puchert M, Rosenstiel P, Wittlieb J, et al. 2013. FoxO is a critical regulator of stem cell maintenance in immortal Hydra. *Ann Neurosci* **109**: 19702.

Bondar T, Medzhitov R. 2010. p53-mediated hematopoietic stem and progenitor cell competition. *Cell Stem Cell* **6**: 309–322.

Booth LN, Brunet A. 2016. The Aging Epigenome. *Mol Cell* **62**: 728–744.

Bourque G, Burns KH, Gehring M, Gorbunova V, Seluanov A, Hammell M, Imbeault M, Izsvák Z, Levin HL, Macfarlan TS, et al. 2018. Ten things you should know about transposable elements. *Genome Biol* **19**: 199.

Boveri T. 1914. *Henry Harris's new translation of Theodor Boveri's 1914 monograph Zur Frage der Entstehung maligner Tumoren Concerning the Origin of Malignant Tumours*. The Company of Biologists and Cold Spring Harbor Laboratory Press.

Brown S, Pineda CM, Xin T, Boucher J, Suozzi KC, Park S, Matte-Martone C, Gonzalez DG, Rytlewski J, Beronja S and Grevo V. 2017. Correction of aberrant growth preserves tissue homeostasis. *Nature* **548**: 334–337.

Brumby AM, Richardson HE. 2003. scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila. - Brumby, Richardson - The EMBO jou.pdf. **22**: 5769–5779.

Brunet A, Rando TA. 2017. Interaction between epigenetic and metabolism in aging stem cells. *Curr Opin Cell Biol* **45**: 1–7.

Buscarlet M, Provost S, Zada YF, Bourgoin V, Mollica L, Dubé MP, Busque L. 2018. Lineage restriction analyses in CHIP indicate myeloid bias for TET2 and multipotent stem cell origin for DNMT3A. *Blood* **132**: 277–280.

Busque BL, Mio R, Mattioli J, Brais E, Blais N, Lalonde Y, Maragh M, Gilliland DG. 1990. Nonrandom X-Inactivation Patterns in Normal Females: Lyonization Ratios Vary With Age. *Blood* 59–66.

Busque L, Patel JP, Figueroa ME, Vasanthakumar A, Provost S, Hamilou Z, Mollica L, Li J, Viale A, Heguy A, et al. 2012. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nat Genet* **44**: 1179–1181.

Busuttil RA, Garcia AM, Reddick RL, Dollé MET, Calder RB, Nelson JE, Vijg J. 2007. Intra-organ variation in age-related mutation accumulation in the mouse. *PLoS One* **2**: 1–10.

Cai. X, Evrony. GD, Lehmann. HS, Elhosary. PS, Mehta. BK, Poduri.A and Walsh, CA. Single-Cell, Genome-wide Sequencing Identifies Clonal Somatic Copy-Number Variation in the Human Brain. Cell Rep. Cell Rep. 2014 September 11; 8(5): 1280–1289. doi:10.1016/j.celrep.2014.07.043

Carrero D, Soria-Valles C, López-Otín C. 2016. Hallmarks of progeroid syndromes: lessons from mice and reprogrammed cells. *Dis Model Mech* **9**: 719–735.

Carvalho CMB, Lupski JR. 2016. Mechanisms underlying structural variant formation in genomic disorders. *Nat Rev Genet* **17**: 224–238.

Cavallucci V, Fidaleo M, Pani G. 2016. Neural Stem Cells and Nutrients: Poised Between Quiescence and Exhaustion. *Trends Endocrinol Metab* **27**: 756–769.

Chandel NS, Jasper H, Ho TT, Passegué E. 2016. Metabolic regulation of stem cell function in tissue homeostasis and organismal ageing. *Nat Cell Biol* **18**: 823–832.

Chen J, Astle CM, Harrison DE. 2000. Genetic regulation of primitive hematopoietic stem cell senescence. *Exp Hematol* **28**: 442–450.

Cho IJ, Lui PPW, Obajdin J, Riccio F, Stroukov W, Willis TL, Spagnoli F, Watt FM. 2019. Mechanisms, Hallmarks, and Implications of Stem Cell Quiescence. *Stem Cell Reports* **12**: 1190–1200.

Choate KA, Lu Y, Zhou J, Choi M, Elias PM, Farhi A, Nelson-Williams C, Crumrine D, Williams ML, Nopper AJ, et al. 2010. Mitotic Recombination in Patients with Ichthyosis Causes Reversion of Dominant Mutations in KRT10. *Science (80-)* **60**: 94–97.

Conboy IH, Conboy MJ, Smythe GM, Rando TA. 2003. Notch-Mediated Restoration of Regenerative Potential to Aged Muscle. *Science (80-)* **302**: 1575–1577.

Coombs CC, Zehir A, Devlin SM, Kishtagari A, Syed A, Jonsson P, Hyman DM, Solit DB, Robson ME, Baselga J, et al. 2017. Therapy-Related Clonal Hematopoiesis in Patients with Non-hematologic Cancers Is Common and Associated with Adverse Clinical Outcomes. *Cell Stem Cell* **21**: 374-382.e4.

Coufal NG, Garcia-perez JL, Peng GE, Yeo GW, Mu Y, Lovci MT, Morell M, Shea KSO, Moran J V, Gage FH. 2009. L1 Retrotransposition in Human Neural Progenitor Cells Nicole. *Nature* **460**: 1127–1131.

De la Cova. C, Abril. M, Bellosta. P, Gallant. P, Johnston. LA. 2004. Drosophila Myc Regulates Organ Size by Inducing Cell Competition. Cell 117: 107–116. doi: 10.1016/S0092-8674(04)00214-4

De Navascués J, Perdigoto CN, Bian Y, Schneider MH, Bardin AJ, Martínez-Arias A, Simons BD. 2012. Drosophila midgut homeostasis involves neutral competition between symmetrically dividing intestinal stem cells. *EMBO J* **31**: 2473–2485.

Dolle MET. 2002. Mutational fingerprints of aging. *Nucleic Acids Res* **30**: 545–549.

Dolle MET, Snyder WK, Gossen JA, Lohman PHM, Vijg J. 2000. Distinct spectra of somatic mutations accumulated with age in mouse heart and small intestine. *PNAS* **97**.

Edifizi D, Nolte H, Babu V, Castells-Roca L, Mueller MM, Brodesser S, Krüger M, Schumacher B. 2017. Multilayered Reprogramming in Response to Persistent DNA Damage in C. elegans. *Cell Rep* **20**: 2026–2043.

Erwin JA, Paquola ACM, Singer T, Gallina I, Novotny M, Quayle C, Bedrosian TA, Alves FIA, Butcher CR, Herdy JR, et al. 2016. L1-associated genomic regions are deleted in somatic cells of the healthy human brain. *Nat Neurosci* **19**: 1583–1591.

Evrony GD, Cai X, Lee E, Hills LB, Elhosary PC, Lehmann HS, Parker JJ, Atabay KD, Gilmore EC,

Poduri A, et al. 2012. Single-neuron sequencing analysis of l1 retrotransposition and somatic mutation in the human brain. *Cell* **151**: 483–496.

Faulkner GJ, Garcia-Perez JL. 2017. L1 Mosaicism in Mammals: Extent, Effects, and Evolution. *Trends Genet* **33**: 802–816.

Fey MF, Liechti-Gallati S, Von Rohr A, Borisch B, Theilkas L, Schneider V, Oestreicher M, Nagel S, Ziemiecki A, Tobler A. 1994. Clonality and X-Inactivation Patterns in Hematopoietic Cell Populations Detected by the Highly Informative M27@ DNA Probe. *Blood*.

Flach J, Bakker ST, Mohrin M, Conroy PC, Pietras EM, Reynaud D, Alvarez S, Diolaiti ME, Ugarte F, Forsberg EC, et al. 2014. Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells. *Nature* **512**: 198–202.

Franco I, Johansson A, Olsson K, Vrtačnik P, Lundin P, Helgadottir HT, Larsson M, Revêchon G, Bosia C, Pagnani A, et al. 2018. Somatic mutagenesis in satellite cells associates with human skeletal muscle aging. *Nat Commun* **9**.

Gan B, Hu J, Jiang S, Liu Y, Sahin E, Zhuang L, Fletcher-Sananikone E, Colla S, Wang YA, Chin L, et al. 2010. Lkb1 regulates quiescence and metabolic homeostasis of haematopoietic stem cells. *Nature* **468**: 701–704.

Ganuza M, Hall T, Finkelstein D, Wang Y-D, Chabot A, Kang G, Bi W, Wu G, McKinney-Freeman S. 2019. The global clonal complexity of the murine blood system declines throughout life and after serial transplantation. *Blood* **133**: blood-2018-09-873059.

Garcia AM, Calder RB, Dollé MET, Lundell M, Kapahi P, Vijg J. 2010. Age- and temperature-dependent somatic mutation accumulation in Drosophila melanogaster. *PLoS Genet* **6**: 27.

Garinis GA, Uittenboogaard LM, Stachelscheid H, Fousteri M, van Ijcken W, Breit TM, van Steeg H, Mullenders LHF, van der Horst GTJ, Brüning JC, et al. 2009. Persistent transcription-blocking DNA lesions trigger somatic growth attenuation associated with longevity. *Nat Cell Biol* **11**: 604–615.

Genovese G, Kähler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, Chambert K, Mick E, Neale BM, Fromer M, et al. 2014. Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence. *N Engl J Med* **371**: 2477–2487.

Giese H, Snyder WK, Van Oostrom C, Van Steeg H, Dollé MET, Vijg J. 2002. Age-related mutation accumulation at a lacZ reporter locus in normal and tumor tissues of Trp53-deficient mice. *Mutat*

Res - Genet Toxicol Environ Mutagen **514**: 153–163.

Gutierrez-Martinez P, Hogdal L, Nagai M, Kruta M, Singh R, Sarosiek K, Nussenzweig A, Beerman I, Letai A, Rossi DJ. 2018. Diminished apoptotic priming and ATM signalling confer a survival advantage onto aged haematopoietic stem cells in response to DNA damage. *Nat Cell Biol*.

Harrison DE. 1978. Loss of proliferative capacity in immunohemopoietic stem cells caused by serial transplantation rather than aging. *J Exp Med* **147**: 1526–1531.

Holstege H, Pfeiffer W, Sie D, Hulsman M, Nicholas TJ, Lee CC, Ross T, Lin J, Miller MA, Ylstra B, et al. 2014. Somatic mutations found in the healthy blood compartment of a 115-yr-old woman demonstrate oligoclonal hematopoiesis. *Genome Res* **24**: 733–742.

Hsieh JCF, Van Den Berg D, Kang H, Hsieh CL, Lieber MR. 2013. Large chromosome deletions, duplications, and gene conversion events accumulate with age in normal human colon crypts. *Aging Cell* **12**: 269–279.

Huang N, Lee I, Marcotte EM, Hurles ME. 2010. Characterising and predicting haploinsufficiency in the human genome. *PLoS Genet* **6**: 1–11.

Inomata K, Aoto T, Binh NT, Okamoto N, Tanimura S, Wakayama T, Iseki S, Hara E, Masunaga T, Shimizu H, et al. 2009. Genotoxic Stress Abrogates Renewal of Melanocyte Stem Cells by Triggering Their Differentiation. *Cell* **137**: 1088–1099.

Issigonis M, Tulina N, De Cuevas M, Brawley C, Sandler L, Matunist E. 2009. JAK-STAT signal inhibition regulates competition in the drosophila testis stem cell niche. *Science (80-)* **326**: 153–156.

Jacobs KB, Yeager M, Zhou W, Wacholder S, Rodriguez-santiago B, Hutchinson A, Deng X, Horner M, Cullen M, Epstein CG, et al. 2012. Detectable clonal mosaicism and its relationship to aging and cancer. *Nat Genet* **44**: 651–658.

Jaiswal S, Ebert BL. 2019. Clonal hematopoiesis in human aging and disease. *Science* **366**.

Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman P V, Mar BG, Coleman Lindsley R, Mermel CH, Burtt N, Chavez A, et al. 2014. Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes NIH Public Access. *N Engl J Med* **371**: 2488–2498.

Janzen V, Forkert R, Fleming HE, Saito Y, Waring MT, Dombkowski DM, Cheng T, DePinho RA, Sharpless NE, Scadden DT. 2006. Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16 INK4a. *Nature* **443**: 421–426.

Jin Z, Kirilly D, Weng C, Kawase E, Song X, Smith S, Schwartz J, Xie T. 2008. Differentiation-Defective Stem Cells Outcompete Normal Stem Cells for Niche Occupancy in the Drosophila Ovary. *Cell Stem Cell* **2**: 39–49.

Jonason A S, Kunala S, Price G J, Restifo R J, Spinelli H M, Persing J A, Leffell D J, Tarone R E, Brash D E. 1996. Frequent clones of p53-mutated keratinocytes in normal human skin. *Proc Natl Acad Sci U S A* **93**: 14025–14029.

Jonkman MF, Scheffer H, Stulp R, Pas HH, Nijenhuis M, Heeres K, Owaribe K, Pulkkinen L, Uitto J. 1997. Revertant mosaicism in epidermolysis bullosa caused by mitotic gene conversion. *Cell* **88**: 543–551.

Kamminga LM, Bystrykh L V, De Boer A, Houwer S, Douma J, Weersing E, Dontje B, De Haan G. 2006. The Polycomb group gene Ezh2 prevents hematopoietic stem cell exhaustion. *Blood* **107**.

Knouse, K.A., Wu, J., Whittaker, C.A., and Amon, A. 2014. Single cell sequencing reveals low levels of aneuploidy across mammalian tissues. Proc. Natl. Acad. Sci. USA 111, 13409–13414. doi: 10.1016/j.cell.2019.03.026

Kolahgar G, Suijkerbuijk SJE, Kucinski I, Poirier EZ, Mansour S, Simons BD, Piddini E. 2015. Cell Competition Modifies Adult Stem Cell and Tissue Population Dynamics in a JAK-STAT-Dependent Manner. *Dev Cell* **34**: 297–309.

Kozar S, Morrissey E, Nicholson AM, van der Heijden M, Zecchini HI, Kemp R, Tavaré S, Vermeulen L, Winton DJ. 2013. Continuous Clonal Labeling Reveals Small Numbers of Functional Stem Cells in Intestinal Crypts and Adenomas. *Cell Stem Cell* **13**: 626–633.

Krishnamurthy J, Ramsey MR, Ligon KL, Torrice C, Koh A, Bonner-Weir S, Sharpless NE. 2006. p16INK4a induces an age-dependent decline in islet regenerative potential. *Nature* **443**: 453–457.

Kuhn HG, Dickinson-Anson H, Gage FH. 1996. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J Neurosci* **16**: 2027–33.

Lahue RS, Au KG, Modrich P. 1989. Mismatch Correction in a Defined System. *Science (80-)* **245**: 1–6.

Laurie CC, Laurie CA, Rice K, Doheny KF, Zelnick LR, Mchugh CP, Ling H, Hetrick KN, Pugh EW, Amos C, et al. 2012. Detectable clonal mosaicism from birth to old age and its relationship to cancer A. *Nat Genet* **44**: 642–650.

Leblond C, Walker B. 1956. Renewal of Cell Populations. *From Dep Anatomy, McGill Univ Montr Canada* 1–9.

Lee-Six H, Olafsson S, Ellis P, Osborne R, Mathijs S, Moore L, Georgakopoulos N, Torrente F, Noorani A, Goddard M, et al. 2019. The landscape of somatic mutation in normal colorectal epithelial cells. *Nature* **574**.

Lee JH, Huynh M, Silhavy JL, Kim S, Dixon-Salazar T, Heiberg A, Scott E, Bafna V, Hill KJ, Collazo A, et al. 2012. De novo somatic mutations in components of the PI3K-AKT3-mTOR pathway cause hemimegalencephaly. *Nat Genet* **44**: 941–5.

Levayer R. 2019. Solid stress, competition for space and cancer: the opposing roles of mechanical cell competition in tumour initiation and growth. *Semin Cancer Biol*.

Li Z, Cai X, Cai C, Wang J, Zhang W, Petersen BE, Yang F. 2011. Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. **118**: 4509–4519.

Li Z, Woo CJ, Iglesias-Ussel MD, Ronai D, Scharff MD. 2004. The generation of antibody diversity through somatic hypermutation and class switch recombination. *Genes Dev* **18**: 1–11.

Lindahl T. 1974. An N-glycosidase from Escherichia coli that releases free uracil from DNA containing deaminated cytosine residues. *Proc Natl Acad Sci U S A* **71**: 3649–53.

Lindahl T, Barnes DE. 2000. Repair of Endogenous DNA Damage. *Cold Spring Harb Symp Quant Biol* **65**: 127–134.

Lindahl T, Nyberg B. 1972. Rate of Depurination of Native Deoxyribonucleic Acid. *Biochemistry* **11**: 3610–3618.

Lindhurst MJ, Parker VER, Payne F, Sapp JC, Rudge S, Harris J, Witkowski AM, Zhang Q, Groeneveld MP, Scott CE, et al. 2012. Mosaic overgrowth with fibroadipose hyperplasia is caused by somatic activating mutations in PIK3CA. *Nat Genet* **44**: 928–933.

Liu N, Matsumura H, Kato T, Ichinose S, Takada A, Namiki T, Asakawa K, Morinaga H, Mohri Y, De Arcangelis A, et al. 2019. Stem cell competition orchestrates skin homeostasis and ageing. *Nature*.

Lodato MA, Barton AR, Yandava CN, Woodworth MB, Yang P, Walsh CA, Park PJ, Sherman MA, Hatem NE, Kwon M, et al. 2018. Aging and neurodegeneration are associated with increased mutations in single human neurons. *Science (80-)* **359**: 555–559.

Lodato MA, Woodworth MB, Lee S, Evrony GD, Mehta BK, Karger A, Chittenden TW, D'Gama AM, Cai X, Luquette LJ, et al. 2015. Somatic mutation in single human neurons tracks developmental and transcriptional history. *Science (80-)* **350**: 94–98.

Lopez-Garcia C, Klein AM, Simons BD, Douglas †, Winton J. 2010. Intestinal Stem Cell Replacement Follows a Pattern of Neutral Drift. *Science (80-)* **330**.

MacRae SL, Croken MM, Calder RB, Aliper A, Milholland B, White RR, Zhavoronkov A, Gladyshev VN, Seluanov A, Gorbunova V, et al. 2015. DNA repair in species with extreme lifespan differences. *Aging (Albany NY)* **7**: 1171–84.

Martin N, Beach D. 2014. Ageing as developmental decay : *Trends Mol Med* **20**: 1–8.

Martincorena I, Fowler JC, Wabik A, Lawson ARJ, Abascal F, Hall MWJ, Cagan A, Murai K, Mahbubani K, Stratton MR, et al. 2018a. Somatic mutant clones colonize the human esophagus with age. **3879**: 1–14.

Martincorena I, Fowler JC, Wabik A, Lawson ARJ, Abascal F, Hall MWJ, Cagan A, Murai K, Mahbubani K, Stratton MR, et al. 2018b. Somatic mutant clones colonize the human esophagus with age. *Science*.

Martincorena I, Fowler JC, Wabik A, Lawson ARJ, Abascal F, Hall MWJ, Cagan A, Murai K, Mahbubani K, Stratton MR, et al. 2018c. Supplementary Materials for. 1–39.

Martincorena I, McLaren S, Tubio JM, Campbell PJ, Van Loo P, Alexandrov LB, Jones PH, Fullam A, Roshan A, Widaa S, et al. 2015. High burden and pervasive positive selection of somatic mutations in normal human skin. *Science (80-)* **348**: 880–886.

Martincorena I, Raine KM, Gerstung M, Dawson KJ, Haase K, Van Loo P, Davies H, Stratton MR, Campbell PJ. 2017. Universal Patterns of Selection in Cancer and Somatic Tissues. *Cell*.

Matsumura H, Mohri Y, Thanh Binh N, Morinaga H, Fukuda M, Ito M, Kurata S, Hoeijmakers J, Nishimura EK. 2016. Stem cells: Hair follicle aging is driven by transepidermal elimination of stem cells via COL17A1 proteolysis. *Science (80-)* **351**.

McClintock B. 1950. The Origin and Behaviour of Mutable Loci in Maize. *Proc Natl Acad Sci* **36**: 345–355.

McConnell. MJ, Lindberg. MR, Brennand. KJ, Julia, CP, Voet. T, Cowing-Zitron. C, Shumilina.S, Lasken. RS, Vermeesch. J, Ira. MH, Gage, FH. Mosaic Copy Number Variation in human neurons. Science. 1; 342(6158): 632–637. doi:10.1126/science.1243472

McKerrell T, Park N, Moreno T, Grove CS, Ponstingl H, Stephens J, Crawley C, Craig J, Scott MA, Hodkinson C, et al. 2015. Leukemia-Associated Somatic Mutations Drive Distinct Patterns of Age-Related Clonal Hemopoiesis. *Cell Rep* **10**: 1239–1245.

Merritt AJ, Potten CS, Watson AJM, Loh DY, Nakayama K, Hickman JA. 1995. Differential expression of bcl-2 in intestinal epithelia Correlation with attenuation of apoptosis in colonic crypts and the incidence of colonic. **2271**: 2261–2271.

Mohrin M, Bourke E, Alexander D, Warr MR, Barry-holson K, Beau MM Le, Morrison CG, Passegué E. 2011. Repair and Mutagenesis. *Cell Stem Cell* **7**: 174–185.

Molofsky A V., Slutsky SG, Joseph NM, He S, Pardal R, Krishnamurthy J, Sharpless NE, Morrison SJ. 2006. Increasing p16INK4a expression decreases forebrain progenitors and neurogenesis during ageing. *Nature* **443**: 448–452.

Morata G, Ripoll P. 1975. *Minutes: Mutants of Drosophila Autonomously Affecting Cell Division Rate*.

Moreno E, Basler K. 2004. dMyc Transforms Cells into Super-Competitors developing tissue remains unchanged and no morpho. *Cell* **117**: 117–129.

Morrison SJ, Wandycz AM, Akashi K, Globerson A, Weissman IL. 1996. The aging of hematopoietic stem cells. *Nat Med* **2**: 1011–1016.

Moskalev AA, Shaposhnikov M V., Plyusnina EN, Zhavoronkov A, Budovsky A, Yanai H, Fraifeld VE. 2013. The role of DNA damage and repair in aging through the prism of Koch-like criteria. *Ageing Res Rev* **12**: 661–684.

Mueller MM, Castells-roca L, Babu V, Ermolaeva MA. 2015. Europe PMC Funders Group DAF-16 / FoxO and EGL-27 / GATA promote developmental growth in response to persistent somatic DNA damage. *Nat Cell Biol* **16**: 1168–1179.

Muotri AR, Chu VT, Marchetto MCN, Deng W, Moran J V., Gage FH. 2005. Somatic mosaicism in neuronal precursor cells mediated by L1 retrotransposition. *Nature* **435**: 903–910.

Nakazawa H, English D, Randell PL, Nakazawa K, Martel N, Armstrong BK, Yamasaki H. 1993. UV and skin cancer: specific p53 gene mutation in normal skin as a biologically relevant exposure measurement. *Proc Natl Acad Sci* **91**: 360–364.

Nicholson AM, Olpe C, Hoyle A, Thorsen AS, Rus T, Colombé M, Brunton-Sim R, Kemp R, Marks K, Quirke P, et al. 2018. Fixation and Spread of Somatic Mutations in Adult Human Colonic Epithelium. *Cell Stem Cell* **22**: 909-918.e8.

Niedernhofer LJ, Gurkar AU, Wang Y, Vijg J, Hoeijmakers JHJ, Robbins PD. 2018. Annual Review of Biochemistry Nuclear Genomic Instability and Aging. *Annu Rev Biochem* 295–322.

Nishimura EK, Granter SR, Fisher DE. 2005. Mechanisms of hair graying: Incomplete melanocyte stem cell maintenance in the niche. *Science (80-)* **307**: 720–724.

Nystul T, Spradling A. 2007. An Epithelial Niche in the Drosophila Ovary Undergoes Long-Range Stem Cell Replacement. *Cell Stem Cell* **1**: 277–285.

O'Huallachain M, Karczewski KJ, Weissman SM, Urban AE, Snyder MP. 2012. Extensive genetic variation in somatic human tissues. *Proc Natl Acad Sci U S A* **109**: 18018–23.

Oberdoerffer P, Michan S, McVay M, Mostoslavsky R, Vann J, Park SK, Hartlerode A, Stegmuller J, Hafner A, Loerch P, et al. 2008. SIRT1 Redistribution on Chromatin Promotes Genomic Stability but Alters Gene Expression during Aging. *Cell* **135**: 907–918.

Petruseva IO, Evdokimov AN, Lavrik OI. 2017. Genome Stability Maintenance in Naked. *Acta Naturae* **9**: 31–41.

Poduri A, Evrony GD, Xuyu C, Elhosary PC, Beroukhim R, Lehtinen MK, Hills LB, Heinzen EL, Hill A, Hill RS, et al. 2012. Somatic Activation ofAKT3 Causes Hemispheric Developmental Brain Malformations. *Neuron* **74**: 41–48.

Potten CS, Grant HK. 1998. The relationship between ionizing radiation-induced apoptosis and stem cells in the small and large intestine. *Br J Cancer* **78**: 993–1003.

Razin A, Riggs A. 1980. DNA Methymation and Gene Function. *Science (80-)* **210**.

Rehen SK, McConnell MJ, Kaushal D, Kingsbury MA, Yang AH, Chun J. 2002. Chromosomal variation in neurons of the developing and adult mammalian nervous system. *Proc Natl Acad Sci* **98**: 13361–13366.

Rivière JB, Mirzaa GM, O'Roak BJ, Beddaoui M, Alcantara D, Conway RL, St-Onge J, Schwartzentruber JA, Gripp KW, Nikkel SM, et al. 2012. De novo germline and postzygotic mutations in AKT3, PIK3R2 and PIK3CA cause a spectrum of related megalencephaly syndromes. *Nat Genet* **44**: 934–940.

Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, Weissman IL. 2007. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. *Nature* **447**: 725–729.

Rübe CE, Fricke A, Widmann TA, Fürst T, Madry H, Pfreundschuh M, Rübe C. 2011. Accumulation of DNA damage in hematopoietic stem and progenitor cells during human aging. *PLoS One* **6**.

Sacco A, Mourkioti F, Tran R, Choi J, Llewellyn M, Kraft P, Shkreli M, Delp S, Pomerantz JH, Artandi SE, et al. 2010. Short telomeres and stem cell exhaustion model duchenne muscular dystrophy in mdx/mTR mice. *Cell* **143**: 1059–1071.

Sancar A. 1993. Photobiology school nucleotide excision. **57**: 905–921.

Schaniel C, Sirabella D, Qiu J, Niu X, Lemischka IR, Moore KA. 2011. Wnt-inhibitory factor 1 dysregulation of the bone marrow niche exhausts hematopoietic stem cells. *Blood* **118**: 2420–2429.

Sharpless NE, DePinho RA. 2007. How stem cells age and why this makes us grow old. *Nat Rev Mol Cell Biol* **8**: 703–713.

Singh SK, Singh S, Gadomski S, Sun L, Pfannenstein A, Magidson V, Chen X, Kozlov S, Tessarollo L, Klarmann KD, et al. 2018. Id1 Ablation Protects Hematopoietic Stem Cells from Stress-Induced Exhaustion and Aging. *Cell Stem Cell* **23**: 252-265.e8.

Siudeja K, Bardin AJ. 2017. Somatic recombination in adult tissues: What is there to learn? *Fly (Austin)* **11**: 121–128.

Siudeja K, Nassari S, Gervais L, Skorski P, Lameiras S, Stolfa D, Zande M, Bernard V, Frio TR, Bardin AJ. 2015. Frequent Somatic Mutation in Adult Intestinal Stem Cells Drives Neoplasia and Genetic Mosaicism during Aging. *Cell Stem Cell* **17**: 663–674.

Slaughter P, Southwick W. 1953. “Field cancerization” in oral stratified squamous epithelium. *Cancer* **6**: 1963–1968.

Smith JJ, Timoshevskaya N, Ye C, Holt C, Keinath MC, Parker HJ, Cook ME, Hess JE, Narum SR, Lamanna F, et al. 2018. The sea lamprey germline genome provides insights into programmed genome rearrangement and vertebrate evolution. *Nat Genet* **50**: 270–277.

Snippert HJ, van der Flier LG, Sato T, van Es JH, van den Born M, Kroon-Veenboer C, Barker N, Klein AM, van Rheenen J, Simons BD, et al. 2010. Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell* **143**: 134–144.

Sotiropoulou PA, Candi A, Mascré G, De Clercq S, Youssef KK, Lapouge G, Dahl E, Semeraro C, Denecker G, Marine JC, et al. 2010. Bcl-2 and accelerated DNA repair mediates resistance of hair follicle bulge stem cells to DNA-damage-induced cell death. *Nat Cell Biol* **12**: 572–582.

Sousa-Victor P, Gutarra S, García-Prat L, Rodriguez-Ubreva J, Ortet L, Ruiz-Bonilla V, Jardí M, Ballestar E, González S, Serrano AL, et al. 2014. Geriatric muscle stem cells switch reversible

quiescence into senescence. *Nature* **506**: 316–321.

Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, Boström E, Westerlund I, Vial C, Buchholz BA, et al. 2013. XDynamics of hippocampal neurogenesis in adult humans. *Cell* **153**: 1219.

Staber PB, Zhang P, Ye M, Welner RS, Nombela-Arrieta C, Bach C, Kerenyi M, Bartholdy BA, Zhang H, Alberich-Jordà M, et al. 2013. Sustained PU.1 Levels Balance Cell-Cycle Regulators to Prevent Exhaustion of Adult Hematopoietic Stem Cells. *Mol Cell* **49**: 934–946.

Stern C. 1936. Somatic crossing over and segregation in Drosophila Melanogaster. *Genetics* **21**: 625–730.

Sudo K, Ema H, Morita Y, Nakauchi H. 2000. Age-associated characteristics of murine hematopoietic stem cells. *J Exp Med* **192**: 1273–80.

Tian D, Zumei F, Nancy H, R. Woodrow S, Judy M, David D. 1998. Multifocal accumulation of p53 protein in esophageam carcinoma: Evidence for field cancerization. *Int J Cancer* **575**: 568–575.

Tierney MT, Stec MJ, Rulands S, Simons BD, Sacco A, Jolla L, Program R, Burnham S, Medical P, Jolla L, et al. 2018. to tissue repair and homeostatic aging. *Cell Stem Cell* **22**: 119–127.

Traulsen A, Lenaerts T, Pacheco JM, Dingli D. 2013. On the dynamics of neutral mutations in a mathematical model for a homogeneous stem cell population. *J R Soc Interface* **10**.

Upton KR, Gerhardt DJ, Jesuadian JS, Richardson SR, Sánchez-Luque FJ, Bodea GO, Ewing AD, Salvador-Palomeque C, Van Der Knaap MS, Brennan PM, et al. 2015. Ubiquitous L1 mosaicism in hippocampal neurons. *Cell* **161**: 228–239.

van Velthoven CTJ, Rando TA. 2019. Stem Cell Quiescence: Dynamism, Restraint, and Cellular Idling. *Cell Stem Cell* **24**: 213–225..

Vaughen J, Igaki T. 2016. Slit-Robo Repulsive Signaling Extrudes Tumorigenic Cells from Epithelia. *Dev Cell* **39**: 683–695.

Walter D, Lier A, Geiselhart A, Thalheimer FB, Huntscha S, Sobotta MC, Moehrle B, Brocks D, Bayindir I, Kaschutnig P, et al. 2015. Exit from dormancy provokes DNA-damage-induced attrition in haematopoietic stem cells. *Nature* **520**: 549–552.

Wang J, Davis RE. 2014. Programmed DNA elimination in multicellular organisms. *Curr Opin Genet Dev*.

Welch JS, Ley TJ, Link DC, Miller CA, Larson DE, Koboldt DC, Wartman LD, Lamprecht TL, Liu F,

Xia J, et al. 2012. The origin and evolution of mutations in acute myeloid leukemia. *Cell* **150**: 264–278.

Williams AB, Schumacher B. 2017. DNA damage responses and stress resistance: Concepts from bacterial SOS to metazoan immunity. *Mech Ageing Dev* **165**: 27–32.

Yamamoto M, Ohsawa S, Kunimasa K, Igaki T. 2017. The ligand Sas and its receptor PTP10D drive tumour-suppressive cell competition. *Nature* **542**: 246–250.

Yizhak K, Aguet F, Kim J, Hess J, Kubler K, Grimsby J, Frazer R, Zhang H, Haradhvala N, Rosebrock D, et al. 2019. A comprehensive analysis of RNA sequences reveals macroscopic somatic clonal expansion across normal tissues. *Science (80-)* **364**:

Yokoyama A, Kakiuchi N, Yoshizato tetsuichi, Nannya Y, Suzuki H, takeuchi Y, Shiozawa Y, Sato Y, Aoki K, Ki Kim S, et al. 2019. Age-related remodelling of oesophageal epithelia by mutated cancer drivers. *Nature*.

Yue F, Bi P, Wang C, Li J, Liu X, Kuang S. 2016. Conditional Loss of Pten in Myogenic Progenitors Leads to Postnatal Skeletal Muscle Hypertrophy but Age-Dependent Exhaustion of Satellite Cells. *Cell Rep* **17**: 2340–2353.

Zhang J, Walter JC. 2014. Mechanism and regulation of incisions during DNA interstrand cross-link repair. *DNA Repair (Amst)* **19**: 135–142.

Zhang Y, Kalderon D. 2001. Hedgehog acts as a somatic stem cell factor in the Drosophila ovary. *Nature* **410**: 599–604.

Zink F, Stacey SN, Norddahl GL, Frigge ML, Magnusson OT, Jonsdottir I, Thorgeirsson TE, Sigurdsson A, Gudjonsson SA, Gudmundsson J, et al. 2017. Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. *Blood*.