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REVIEW

Balancing self-renewal against genome preservation in stem cells: How do they manage to have the cake and eat it too?

Robert Y. L. Tsai[1,2]

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Abstract Stem cells are endowed with the awesome power of self-renewal and multi-lineage differentiation that allows them to be major contributors to tissue homeostasis. Owing to their longevity and self-renewal capacity, they are also faced with a higher risk of genomic damage compared to differentiated cells. Damage on the genome, if not prevented or repaired properly, will threaten the survival of stem cells and culminate in organ failure, premature aging, or cancer formation. It is therefore of paramount importance that stem cells remain genomically stable throughout life. Given their unique biological and functional requirement, stem cells are thought to manage genotoxic stress somewhat differently from non-stem cells. The focus of this article is to review the current knowledge on how stem cells escape the barrage of oxidative and replicative DNA damage to stay in self-renewal. A clear statement on this subject should help us better understand tissue regeneration, aging, and cancer.

Keywords Aging · Cancer stem cells · DNA repair · Immortal strand · Nucleostemin · Oxidative stress · Replicative stress · Telomere · Translesion synthesis · Tumor progression

✉ Robert Y. L. Tsai
rtsai@ibt.tamhsc.edu

[1] Center for Translational Cancer Research, Institute of Biosciences and Technology, Texas A&M University Health Science Center, 2121 W. Holcombe Blvd, Houston, TX 77030, USA

[2] Department of Molecular and Cellular Medicine, Texas A&M University Health Science Center, College Station, TX 77843, USA

Abbreviations

ABC	ATP-binding cassette
ALT	Alternative lengthening of telomere
Alt-NHEJ	Alternative non-homologous end-joining
APB	ALT-associated PML bodies
ATM	Ataxia telangiectasia mutated
ATR	Ataxia telangiectasia and Rad3 related
ATRX	Alpha thalassemia/mental retardation syndrome X-linked (also known as RAD54)
BLM	Bloom helicase
BRCA1/2	Breast cancer 1/2
CO-FISH	Chromosome orientation fluorescence in situ hybridization
C-NHEJ	Classical non-homologous end-joining
CtBP	C-terminal binding protein
CtIP	CtBP interacting protein
DDR	DNA damage response
DNA-PKcs	DNA-dependent protein kinase catalytic subunit
DSBs	Double-stranded breaks
ECTR	Extrachromosomal telomere repeats
ERCC1	Excision repair cross-complementation group 1
ERCC4	Excision repair cross-complementation group 4 (also known as XPF)
ES	Embryonic stem
FANCD1	Fanconi anemia complementation group D1
HLTF	Helicase-like transcription factor
HR	Homologous recombination
ICL	Interstrand crosslink
iPS	Induced pluripotent stem
IR	Ionizing radiation
KD	Knock-down
KO	Knock-out
MDR	Multidrug resistance

MRN	MRE11/RAD50/NBS1
MSH2	mutS homolog 2
NS	Nucleostemin
PARP1/2	Poly(ADP)ribose polymerase 1 or 2
PCNA	Proliferating cell nuclear antigen
PML	Promyelocytic leukemia protein
RFC	Replication factor C
RPA	Replication protein A
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SHPRH	SNF2 histone-linker PHD ring helicase
SSA	Single strand annealing
SSBs	Single-stranded breaks
ssDNA	Single-stranded DNA
TERC	Telomerase RNA component
TERT	Telomerase reverse transcriptase
TLS	Translesion synthesis
TopBP1	Topoisomerase II binding protein 1
TRF1	Telomeric repeat factor 1
T-SCE	Telomere sister chromatid exchange
XLF	XRCC4-like factor (also known as Cernunnos)
XRCC1	X-ray repair cross-complementing group 1
WRN	Werner syndrome ATP-dependent helicase

Introduction

Adult animals have developed distinct mechanisms to replenish the inevitable loss of cells that occurs daily as a result of normal attrition or accidental injury. In lower organisms with simple body plan, tissue regeneration may be carried out by some differentiated cells that can be reversed back to an undifferentiated state and then redirected into cell fates other than their original ones. This process is known as de-differentiation. In higher vertebrates, de-differentiation may cause undesired consequences of incomplete or even faulty differentiation due to the changing environment and the complexity of tissue organization. To avoid such aberrancy, a small population of cells with tissue-restricted potential (known as somatic, tissue-specific, or tissue-resident stem cells) are embedded within the organs that require constant renewal. Those tissue-specific stem cells persist throughout life and retain the ability to: (1) become all cell types cognate to their resident tissues (i.e. multipotential), and (2) maintain their population throughout continuous division (i.e. self-renewal) [1]. A vast amount of studies done in the 1990s have concluded that such stem-like cells not only exist in organs that turn over regularly but also can be isolated from those with no apparent regenerative capability, which even include the regeneration-unfriendly neural tissue [2, 3]. This revelation inspires the idea that stem or stem-like cells may be embedded in every organ and rekindled if necessary. So begins the era of stem cells!

The power of stem cells can work as a double-edged sword. On the bright side, self-renewal ensures that stem cell populations are not depleted over time so that they can provide an inexhaustible source for cell replacement in vivo and in therapeutics [4]. To date, scientists are still trying to unleash the de novo regenerative power of stem cells in tissues that are either non-regenerative by nature or capable of regeneration but decompensated by diseases, injuries, or the aging process. On the dark side, the self-renewal-driving machinery may be hijacked by transformed cells to achieve replicative immortality [5]. On this conceptual ground thus rises the cancer stem cell (CSC) theory, which postulates that there are stem-like cells in tumors that are tumorigenic and sit atop the tumor cellular hierarchy [6, 7]. In the 2000s, the existence of CSCs has been extensively researched and experimentally shown in acute myeloid leukemia [8, 9], breast cancers [10], brain tumors [11], and other types of solid tumors [12–16].

Because of their long lifespan and self-renewal properties, normal stem cells and CSCs (hereafter collectively referred to as stem cells unless otherwise specified) are thought to be uniquely equipped to deal with the occurrence and consequence of genomic damage by ways different from short-term dividing or non-dividing cells. This notion starts to gain a stronger foothold when more and more studies are conducted that deepen our understanding of how stem cells balance between self-renewal and genome preservation [17–20]. Indeed, embryonic stem (ES) cells display a lower mutation rate compared to somatic cells despite their robust mitotic activity [21]. In support, it has been demonstrated that mice deficient in one or more components in the DNA repair pathways, such as ataxia telangiectasia mutated (ATM), DNA ligase 4 (LIG4) [22], DNA-dependent protein kinase catalytic subunit (DNA-PKcs), mutS homolog 2 (MSH2), and Fanconi anemia complementation group D1 (FANCD1), show limited stem cell functions in various tissues [23–32]. Although the exact mechanisms by which stem cells preserve their genome integrity throughout self-renewal may not be entirely clear as yet, I believe that a focused review on this subject will help gather the much needed interest and momentum to this field to inspire new research directions in the future. This article will take on this task from four broad angles that cover damage prevention, stalled replication restart, damage repair, and outcome selection in stem versus non-stem cells (Fig. 1). It will not emphasize as much on how perturbation of these various pathways may affect stem cell functions as on how stem cells differ from other dividing cells in their ways of dealing with genomic stress.

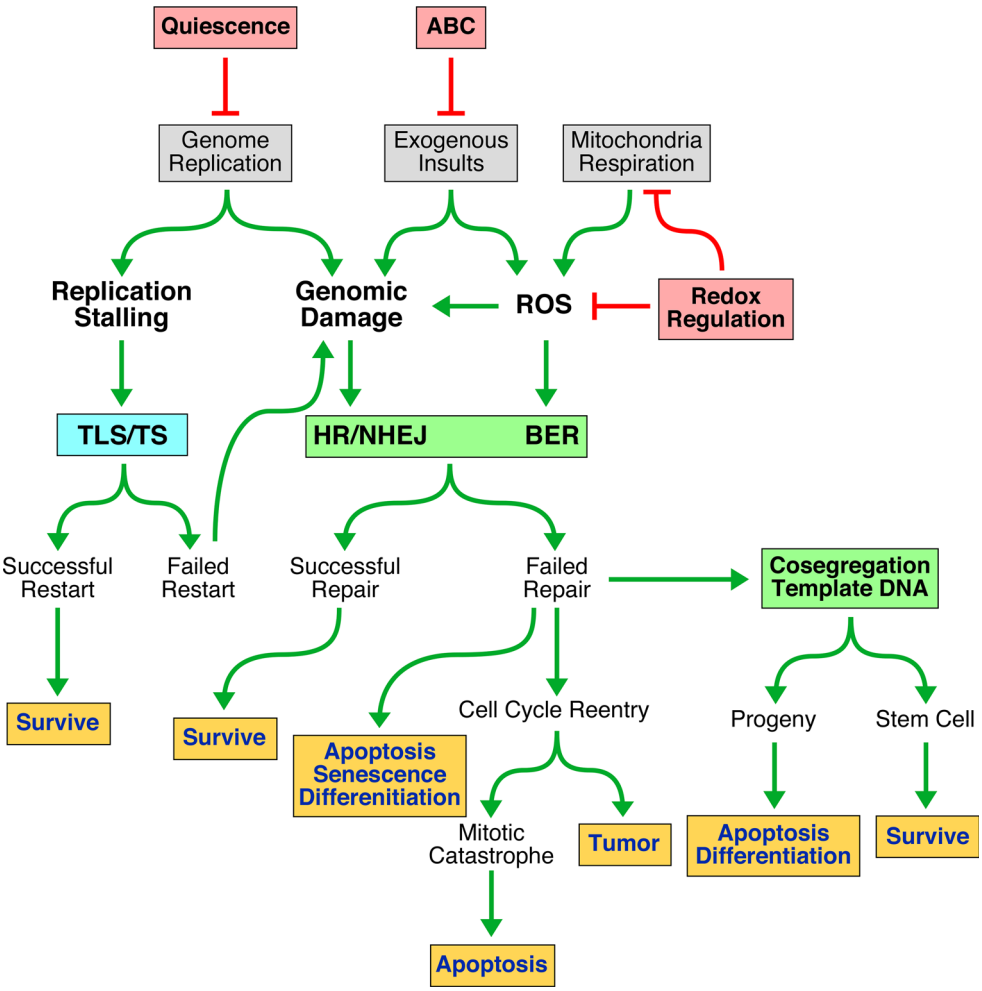

Fig. 1 Genomic stress management in stem cells. DNA damage can occur spontaneously as a result of genome replication and cell metabolism or reactively to exogenous insults (*gray boxes*). Avoiding genomic damage and instability is critical for stem cells to maintain their longevity and self-renewal potential. Stem cells may achieve this goal by employing four strategies that: (1) prevent damage at first sight (*pink boxes*); (2) restart stalled replication forks (*blue box*); (3) repair/cosegregate genomic damage (*green boxes*); or (4) select the least harmful outcome out of many (*yellow boxes*). The *green* and *red arrows* indicate a causative effect or a suppressive effect, respectively. *ABC* ATP-binding cassette transporter, *ROS* reactive oxygen species, *TLS* translesion synthesis, *TS* template switching, *HR* homologous recombination, *NHEJ* non-homologous end-joining, *BER* base excision repair

Nip in the bud: controlling damage at first sight

No cell can avoid the risk of genotoxic damage, especially those that enjoy the benefit of a long and productive life. Not only is the genome under constant attack from extrinsic sources of insults, but it is also faced with damages that arise internally as a result of genome replication, hydrolytic cleavage, which causes DNA deamination or depurination, or reaction to reactive oxygen, nitrogen, or carbonyl species produced during mitochondrial respiration [33]. It is estimated that there may be up to 10^6 DNA damage events occurring in a single cell on a daily basis [34]. The two major cell-extrinsic sources of genome-damaging insults are chemoreagents and ultraviolet (UV) radiation. It has been shown that stem cells express higher levels of ATP-binding cassette (ABC) transporters or multidrug resistance (MDR) genes, which pump out intracellular drugs and lower their amounts inside the cell [35]. So far, there is no evidence indicating that stem cells are less likely exposed to UV or ionizing radiation (IR) than their neighboring non-stem cells.

Genome replication itself is an intrinsic source of double-stranded breaks (DSBs). Replicative DNA damage can happen as a result of three naturally occurring events. First of all, the movement of the replication machinery (also known as the replisome) may be stalled at the sites that are: (1) previously damaged and unrepaired, (2) forming complex secondary structure, (3) bound by protein complexes, or (4) containing fragile DNA or repeat sequences (e.g. ribosomal DNA, telomere, and Alu). It may also be triggered by exogenous chemicals or drugs that block the activity of DNA replication machinery or deplete the endogenous nucleotide pool [36–38]. Stalled replication forks, if unresolved in time, may collapse and result in DSBs. Alternatively, DSBs may be generated spontaneously during genome replication when replisomes encounter single-stranded breaks (SSBs). Lastly, replication of the chromosomal ends will introduce telomere attrition as a result of the end-replication problem. As a repeat sequence itself, the telomere is also subjected to DNA damage during the genome replication process. Mitotic quiescence offers stem cells the first line of defense

against issues arising from genome replication [39]. Many adult tissues harbor a subset of stem cells held in a mitotically quiescent state by their microenvironment for most of the time. However, mitotic quiescence is not a one-size-fits-all solution. Embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, embryonic tissue-specific stem cells, some adult tissue-specific stem cells during injury, and CSCs undergo active self-renewal. Mouse ES cells, in particular, have a shortened G1 phase and an inactive G1/S checkpoint control [40]. Similar cell cycle profiles have also been observed in human ES cells and iPS cells [41]. Consequently, a majority (50–70 %) of these pluripotent stem cells are in the S-phase and hence are susceptible to replication-induced DNA damage [40]. For those cells, a second or third line of defense is needed.

Oxidative stress is another endogenous source of genotoxic insult and is the leading cause of DNA damage in quiescent stem cells. Quiescent stem cells exist in some adult tissues (e.g. hematopoietic stem cells in the bone marrow, bulge stem cells in the hair follicle, and crypt stem cells in the intestinal epithelium), remain mitotically inactive, and undergo asymmetric cell division only when needed. As quiescent stem cells remain largely in the G0 phase, their genome is exempt from replicative DNA damage. Despite that, they are still faced with oxidative stress produced by endogenous mitochondrial respiration and exposure to exogenous UV or chemicals. It is estimated that reactive oxygen species (ROS), which include superoxide radicals, hydroxyl free radicals, and hydrogen peroxide, damage 10^4 bases per day in a human cell [42]. Oxidative stress can create oxidized nucleotides (e.g. 8-oxyguanine), SSBs, and DNA hydrolysis (which leads to abasic or deaminated lesions). It has been shown that ROS have a profound effect in limiting the lifespan of hematopoietic stem cells [43–45]. Fortunately, the ROS level in hematopoietic stem cells is 100-fold less than that in myeloid progenitors. Reduction of ROS in stem cells may be controlled by their high levels of FoxO transcription factors, which operate downstream of the PI3K-AKT pathway and regulate the expression of ROS detoxication genes, including superoxide dismutase 2 (SOD2) and catalase [46–49]. In consistence, the FoxO pathway is required for the maintenance of leukemic CSCs in chronic myeloid leukemia [50]. Bmi1 (a polycomb RING-finger protein) can also help to reduce the generation of ROS from the inside of stem cells [51]. Other than those cell-intrinsic programs, cell-extrinsic factors may play a role as well. Some stem cells are known to reside in a hypoxic microenvironment. The hypoxic stem cell niche has been shown for hematopoietic stem cells [52, 53], intestinal stem cells [54], and breast CSCs [55], and supported by the low oxygen culture condition for neural and hematopoietic stem cells [56, 57]. Within the hypoxic niches, stem cells employ anaerobic glycolysis instead of mitochondrial oxidative phosphorylation for energy metabolism—a decision that may be driven by their low mitochondrial mass and a HIF-1α-controlled mechanism [58–61]. The preferential use of a selective energy pathway also helps to lower the intracellular ROS level of stem cells. However, some studies seem to contradict the link between stem cells and hypoxia. One study showed that the self-renewal of proliferating neural stem cells is propelled by a high ROS level [62]. Another study showed that breast CSCs contain abundant mitochondria, and the enrichment of mitochondria in breast CSCs is driven by the Wnt1/FGF3 pathway [63]. Counter-intuitively, high mitochondrial mass appears to promote the resistance of breast CSCs to DNA damage [64].

A window of opportunity: restarting stalled replication forks before they collapse

As prolonged replication stalling may lead to replication fork collapse, DSBs, and chromosomal rearrangement, all dividing cells must learn how to resolve replication stalling efficiently as their next line of defense [65, 66]. The resolution of stalled replication forks consists of a sensing step and a bypass step. To date, the molecular mechanisms underlying each of these two events have just begun to emerge. As a result, it has yet to be determined whether and how these events operate in a stem cell-specific setting. For this reason, this review will discuss the current knowledge on how cells in general manage to restart the stalled replication fork, with the anticipation that stem cell-unique regulation on some of the pathways may be uncovered in the future.

Sensing replication stalling

Replication fork stalling takes place routinely during genome replication [67, 68]. To finish self-renewal and preserve genome integrity efficiently, mitotically active stem cells need to learn how to reinitiate or bypass the stalled site when they encounter one. Failure to do so may result in the collapse of replication forks that ultimately leads to DSBs. The signaling cascade triggered by replication stalling begins with the uncoupling of DNA polymerase and helicase and the formation of replication protein A (RPA)-coated single-stranded DNAs (ssDNA). DNA-bound RPA then recruits the ataxia telangiectasia and Rad3 related (ATR), Rad17-Rfc2-5, the 9-1-1 clamp, and topoisomerase II binding protein 1 (TopBP1) to the stalled replication site, which serves the function of triggering G2/M arrest and stabilizing the stalled replication fork [69–72]. TopBP1 also plays a role in DNA replication

initiation and is needed for neural progenitors to maintain their genome integrity and reduce replication-associated DNA damage during neural development [73]. Although not completely characterized as yet, the mechanisms by which cells reengage the stalled replication machinery, bypass the lesion site, and restart processive replication can follow either an error-prone translesion synthesis (TLS) pathway or an error-free template-switching pathway [74].

Translesion synthesis (TLS)

Compared to the replicative DNA polymerases [e.g. Pol δ (delta)], those specialized in TLS [e.g. Pol η (eta) and Pol ζ (zeta)] have broader active sites, exhibit lower processivity, and lack 3′-to-5′ exonuclease editing. The type of TLS polymerases recruited to the stalled site will also determine the bypass fidelity. For example, UV-induced pyrimidine dimers can be bypassed by using Pol η in a relatively error-free mode or by using Pol ζ and Rev1 in an error-prone mode [75–77]. A recent study showed that ovarian CSCs express high levels of Pol η, which may contribute to their cisplatin resistance [78]. The TLS mechanism can be viewed as a cycle of DNA polymerase switching, orchestrated by the monoubiquitinylation and deubiquitinylation of proliferating cell nuclear antigen (PCNA) (Fig. 2). During regular DNA replication, PCNA works as a sliding clamp around DNAs and plays an important role in the switching of replicative polymerase from the primase-Pol α [alpha] complex to the processive Pol δ. The DNA loading and unloading of PCNA is controlled by the arc-shaped replication factor C (RFC) complex (Rfc1-5) [79]. At the UV-damaged site, PCNA is monoubiquitinylated on K164 by the RAD6 (E2) and RAD18 (E3) heterodimer [80–83]. Monoubiquitinylated PCNA promotes direct lesion bypass by recruiting TLS polymerases (Pol η and Pol ζ) to the stalled replication fork through the ubiquitin-binding domain present on all Y-family polymerases [84–88]. In addition to PCNA monoubiquitination, Pol η can also be recruited to the UV-damaged site by its direct interaction with RAD18 [85]. Switching between different polymerases allows cells to use Pol η to add the first adenine across the TT dimer, Pol ζ to extend the mismatch, and Pol δ to continue with the rest of DNA replication. Another molecule involved in TLS is C1orf24. C1orf24 is a PCNA-binding protein that stabilizes RAD18 localization, promotes PCNA monoubiquitinylation, and performs polymerase switching from Pol δ to Pol η at the UV-induced DNA damage site by a Valosin-containing protein-dependent mechanism [89]. BAF180 also participates in TLS. BAF180 is the human ortholog of yeast RSC1–RSC2–RSC4 fusion and a component in the chromatin-remodeling complex that consists of BAF57, BAF200, and BRG1 (SWI/SNF core complex) [90]. Depletion of BAF180 has been shown to reduce PCNA ubiquitinylation as well as the chromatin-bound unmodified PCNA after UV radiation. As depletion of BAF180 does not diminish chromatin-bound RPA, it may promote the bypass by remodeling the chromatin structure to support the switching of TLS polymerases and the repriming of PCNA.

Two regulatory events have been reported that negatively control TLS. One involves FbH1, an UvrD DNA repair helicase and the human functional analog of Srs2. It was shown that FbH1 overexpression weakens blocked replication-induced homologous recombination (HR) and reduces nuclear RAD51 foci, suggesting that FbH1 may prevent HR repair by restraining RAD51 localization at the stalled replication site [91]. Moreover, FbH1-deficient cells are hyposensitive to replication stress induced by hydroxyurea, show a reduced activation of ATM and p53, and exhibit better survival with decreased apoptosis [92, 93]. The negative function of FbH1 at the stalled replication site is regulated by its interaction with RPA and PCNA [93, 94] as well as by the impaired recruitment of Pol η to UV-damaged chromatin [94]. Another negative regulator of TLS is the SUMOylation of PCNA. It has been shown that SUMO modification of yeast PCNA negatively affects HR by granting access to the Srs2 helicase (the functional equivalent of human FbH1) to disrupt the RAD51 nucleoprotein filament [95] and by interfering with Eco1-dependent sister chromatid cohesion [96]. In human cells, SUMO modification of PCNA is facilitated by RFC and functions to prevent replication fork collapse into DSBs [97]. If the replication fork stalls at DNA lesion sites, SUMOylated PCNA exhibits the ability to inhibit HR repair [97].

Template switching

Stalled replication can be restarted by an error-free mechanism that involves the polyubiquitinylation of PCNA and template switching. Addition of K63-linked polyubiquitin chains on PCNA requires RAD5 and the MMS2–UBC13 complex, but may or may not take place directly on the monoubiquitinylated PCNA [98, 99]. RAD5 is a member of the SWI/SNF family. It interacts with a heterodimeric E2 enzyme, MMS2–UBC13, to promote methyl methanesulfonate-induced PCNA polyubiquitinylation [100, 101]. Human RAD5 homolog, SNF2 histone-linker PHD ring helicase (SHPRH), is located on chromosome 6q24 and acts as a tumor suppressor in addition to its E2 ubiquitin ligase role [102]. RAD5 also works with RAD6–RAD18 to promote PCNA monoubiquitinylation. Helicase-like transcription factor (HLTF) is another human RAD5 homolog that shares similar domains and functions with SHPRH in binding UBC13 and PCNA and facilitating PCNA polyubiquitinylation [103, 104]. Inactivation of SHPRH or HLTF elevates spontaneous mutagenesis and

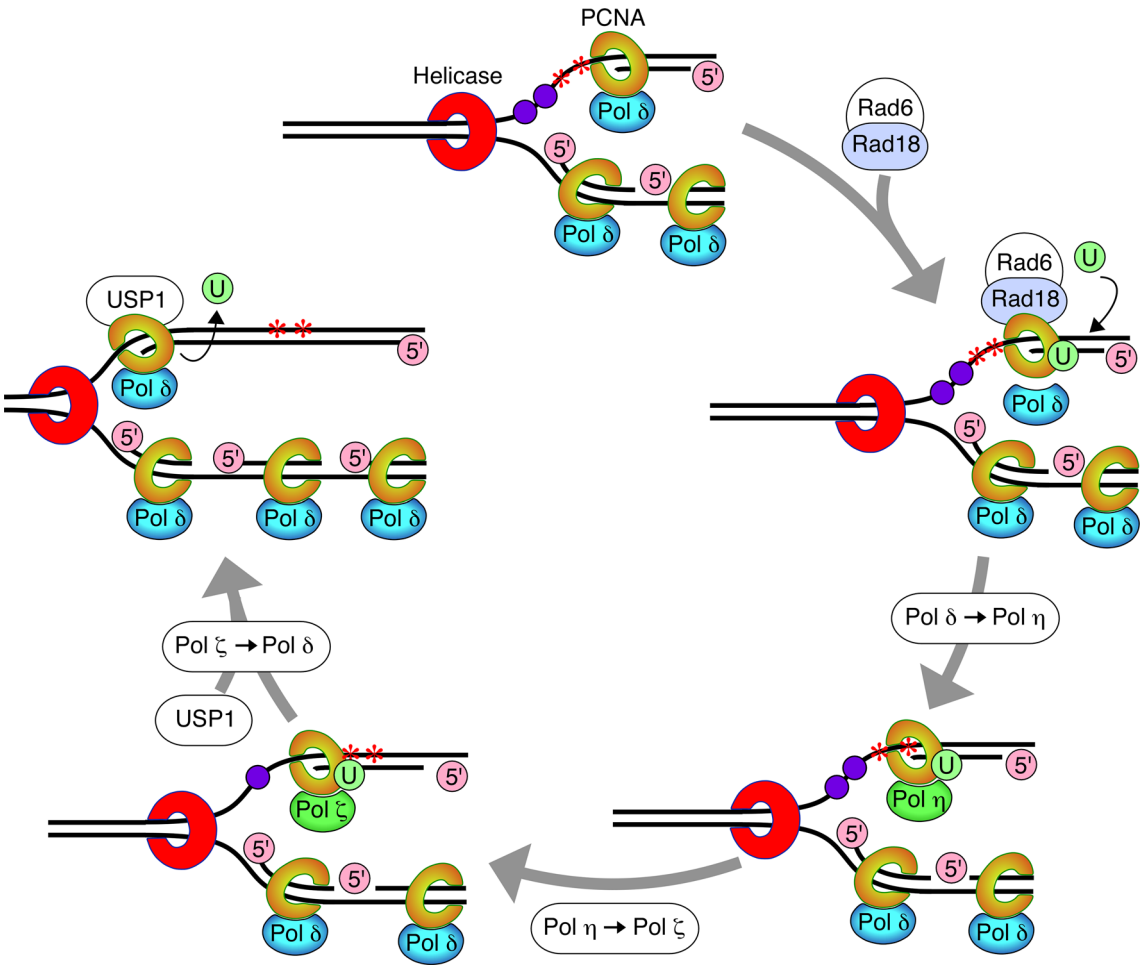

Fig. 2 Translesion synthesis (TLS) represents a cycle of polymerase switching driven by the ubiquitinylation of PCNA. TLS allows replication forks to bypass the stalled sites. The key event underlying TLS is the monoubiquitinylation of PCNA, mediated by Rad6 and Rad18. Monoubiquitinylation of PCNA and its deubiquitinylation by USP1 trigger a cycle of polymerase (Pol) switching between the processive Pol δ and the permissive Pol η and Pol ζ. *Red asterisks* mutations, *U* monoubiquitinylation, *5′* primase/RNAs, *purple circles* replication protein A (RPA)

genome instability [103, 105]. Hence, SHPRH and HLTF cooperatively regulate PCNA polyubiquitinylation to activate the template-switching pathway to protect the replicating genome from DNA lesion-associated mutagenesis, genome instability, and subsequent carcinogenesis. The K164 mutation on PCNA impairs the Pol η- and Pol ζ-dependent TLS defense against UV lesions, and yet imparts a higher RAD51-mediated recombination activity [106]. These findings support that K164 ubiquitinylation is a critical posttranslational modification of PCNA that determines which of the two PPR pathways, i.e. TLS and template switching, will be chosen. Finally, how polyubiquitinylated PCNA turns on the template-switching pathway still remains speculative. One hypothesis is that PCNA polyubiquitinylation may induce template switching via a recombination-like mechanism. In mammalian cells, DSBs and other lesions associated with DNA replication are, for the most part, repaired by HR [107, 108]. It has been shown that RAD51 deficiency can also lead to the accumulation of DSBs at the sites of stalled replication forks, suggesting that RAD51-mediated HR may help resolve the stalling of replication forks [109]. The HR mechanism required for repairing two-ended DSBs has been extensively researched in the past. In contrast, the HR event occurring in response to replication stalling in mammalian cells is different from that seen in the two-ended DSB repair and is much less understood [65, 110, 111].

Better late than never: repairing damaged chromosomes

Base excision repair (BER)

Oxidized nucleotides are removed by the BER mechanism, which corrects oxidized and alkylated bases as well as

SSBs. The BER pathway may work through a short-patch mechanism, which replaces single nucleotides, or a long-patch pathway, which replaces 2–13 nucleotides [112]. BER is initiated by one of several DNA glycosylases [e.g. OGG1 (8-oxoguanine glycosylase), UDG (uracil DNA glycosylase), and AAG (3-alkyladenine DNA glycosylase)] that recognizes and removes specific modified bases. For example, OGG1 is specialized in recognizing 8-oxoG, one of the most common lesions caused by oxidative damage. The resulting abasic or apyrimidinic/apurinic (AP) lesions are then excised by AP endonuclease (e.g. APE1) to create SSB. As an intermediate product of BER, SSB is recognized by poly(ADP-ribose) polymerase 1 (PARP1), which participates in the subsequent recruitment of Pol β [beta] and XRCC1-DNA ligase 3 for gap filling and closure, respectively. It has been shown that human ES cells in general show higher expression levels of BER genes, such as OGG1 and APE1, and that they also exhibit more efficient BER and lower 8-oxoG lesions compared to differentiated cells [113, 114]. Similarly, mouse neural stem and progenitor cells express higher levels of OGG1 and Neil1 than do differentiated neurons [115]. Some BER genes, such as XRCC1, DNA ligase 3, and DNA ligase 1, were found to be down-regulated during the differentiation of mouse myoblasts [116].

Mismatch repair (MMR), nucleotide excision repair (NER), and Fanconi anemia (FA)

The pathway that negotiates base mismatch, insertion loops, and deletion loops created during genome replication is MMR. In this pathway, mismatched bases are sensed by the MSH2–MSH3 or MSH2–MSH6 complex. The MSH complex then recruits MLH-1 and PMS2 to coordinate mismatch removal by exonuclease, gap filling by DNA polymerase, and gap closure by DNA ligase [117]. It has been shown that human pluripotent stem cells again express higher expression levels of MMR-related genes (e.g. MSH2, MSH5, MSH6, MLH-1, and PMS2) and display more efficient MMR repair compared to differentiated cells [113, 114, 118, 119]. UV radiation, environmental pollutants (e.g. aldehydes), and cross-linking reagents (e.g. platinum-related chemotherapeutic agents) can cause helix-distorting DNA lesions. This type of lesions requires the NER pathway for repair [120]. NER can be carried out by a global genome repair (GGR) mechanism, which senses and repairs damage occurring throughout the entire genome and depends on the functions of XPA and XPC, or by a transcription-coupled repair (TCR) mechanism, which repairs lesions on the transcribed strands of transcriptionally active genes and depends on the functions of XPA, Cockayne syndrome A (CSA), and Cockayne syndrome B (CSB) proteins. NER involves the sequential recruitment of a group of proteins that sense and prepare the DNA lesion [i.e. XPA, XPC-RAD23B, CSA, CSB, and transcription factor IIH (TFIIH, including XPB and XPD helicases)], remove damaged nucleotides (i.e. ERCC1-XPF, and XPG), synthesize DNAs (i.e. Pol δ, Pol ε, and accessory proteins such as PCNA and RPA), and close the gap (i.e. DNA ligase 3 and 1) [121–123]. The GGR activity is found to be attenuated upon differentiation of neural and macrophage precursors, whereas the TCR activity remains unchanged during the differentiation of these cells [124, 125]. Another mechanism specialized in the repair of intrastrand crosslinks is the FA pathway. The detail of this pathway can be found in several published reviews, in which readers may find more detail information [126–128]. For the interest of this review, it is worth noting that most of the components in the FA pathway are decreased during macrophage differentiation [129]. Interstrand crosslinks (ICLs), on the other hand, are caused by platinum-related chemotherapeutic agents and mitomycin C. They are repaired by a combination of pathways that include NER, HR, TLS, and FA, and, therefore, will have the same stem cell connotation as described previously [130].

DSB repair choices

Double-stranded breaks can be triggered by exogenous insults and by prolonged replication stalling. Prolonged stalling causes replication forks to collapse into DSBs. In addition, any unrepaired SSB, regardless of its origin, will be converted to a DSB at the replication fork during genome replication. DSBs can lead to chromosomal loss or rearrangement, and is the most lethal threat to all dividing cells. The response of cells to DSBs begins with the recruitment of ataxia telangiectasia mutated (ATM) that eventually turns on one of the four DSB repair programs: classical NHEJ (C-NHEJ), HR, single strand annealing (SSA), and alternative NHEJ (Alt-NHEJ) (Fig. 3). The HR and SSR repair mechanisms involve the pairing of extended homologous sequences and hence take place during the S and G2 phase. Alt-NHEJ may engage microhomology between DSB ends and take place during the S/G2 phase as well. In contrast, C-NHEJ is the predominant mechanism of repair in the G0 and G1 phase but can also operate in the S and G2 phase. According to this cell cycle-dependent preference, mitotically quiescent stem cells in adult tissues use primarily the error-prone C-NHEJ as their pathway of choice for DSB repair, whereas mitotically active stem cells (e.g. ES cells, iPS cells, embryonic tissue-specific stem cells, regenerating adult stem cells, and CSCs) may use all four mechanisms. This cell cycle phase-based selection of DSB repair pathways is consistent with the developmental transition from an HR-based repair in embryos to a C-NHEJ-based repair in adult animals [131–

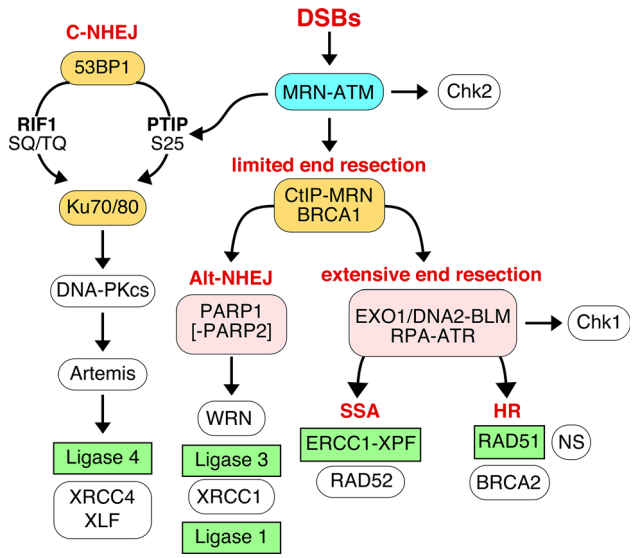

Fig. 3 Four pathways for double-stranded break (DSB) repair. Nascent DSBs are first recognized by ATM and the MRN complex that initiate a series of DNA damage response events that aim at arresting cell cycle and recruiting DSB repair proteins. The C-NHEJ pathway is triggered by direct binding of Ku70/80 protein to DSB ends, followed by the recruitment of DNA-PKcs, Artemis, DNA ligase 4, XRCC4, and XLF. For cells in the S and G2 phase, DSBs can undergo limited end resection, followed by an extended end resection that leads to primarily HR repair but sometimes SSA repair. HR repair requires a core factor, RAD51, and several cofactors, including RAD51 paralogues, BRCA2, and nucleostemin (NS). Alternatively, the exposed 3′ ends of DSBs may be directly ligated by an Alt-NHEJ mechanism that is initiated by PARP1 and followed through by WRN, DNA ligase 3/XRCC1, and DNA ligase1. See text for more abbreviations

133]. In S-phase cells, HR and C-NHEJ appear to compete against each other for DSB sites, but the balance between them differs widely between different species as well as between different cell types of the same species. The key event that decides HR over C-NHEJ is the resection of DSB ends, which involves an initial limited resection step, mediated by the complex of C-terminal binding protein (CtBP) interacting protein (CtIP) and MRN (MRE11, RAD50, and NBS1), and a second extensive resection step, mediated by the EXO1-Bloom helicase (BLM) complex or the DNA2-BLM complex. Recent studies show that C-NHEJ is stimulated by 53BP1 and RIF1, and that HR and DNA end resection are promoted by breast cancer 1 (BRCA1) and CtIP [134–137]. Another mechanism by which haploid yeasts up-regulate C-NHEJ and down-regulate HR (or vice versa in diploid yeasts) is through a MAT-dependent regulation of Nej1. In some instances, cells may also use p53 to choose between HR and C-NHEJ. For example, when rapid cell division is required, p53 suppression may serve the role of suppressing HR as well as preventing cell cycle arrest in favor of a p53-independent apoptotic pathway to get rid of cells with damaged genome. Notwithstanding the pathway choice, the abilities to repair DNA damage by either HR or C-NHEJ are both critically important for the maintenance of the stem cell population as a whole. Their individual importance is further described as follows.

C-NHEJ

Unprocessed DSBs can be directly bound by Ku70/80 and repaired by the C-NHEJ mechanism, which involves an orderly recruitment of DNA-dependent protein kinase catalytic subunit (DNA-PKcs), Artemis, DNA ligase 4, XRCC4, and XLF. C-NHEJ can operate throughout the cell cycle and therefore is the major pathway for DSB repair in quiescent stem cells [138]. However, depending on the extent of end-processing and the fidelity of end-pairing, C-NHEJ-based repair may result in mutations or chromosomal rearrangement and therefore is considered error-prone in a relative sense. For this reason, while the quiescent state of stem cells help minimize their chance of incurring replicative and oxidative DNA damage, it also subjects them to the error-prone C-NHEJ repair mechanism instead of the error-free HR repair mechanism. Genes involved in the C-NHEJ repair are by and large increased in pluripotent stem cells, such as ES cells and iPS cells [113, 114, 139]. However, at least one of the C-NHEJ factors, DNA-PKcs, was shown to be down-regulated in ES cells compared to differentiated cells [133]. Unlike pluripotent stem cells, it was reported that bulge stem cells in the hair follicle exhibit a higher efficiency in C-NHEJ repair of DNA damage compared to epidermal cells as a result of their higher nuclear expression of DNA-PKcs [140]. Similarly, it was shown that thrombopoietin can promote C-NHEJ repair in hematopoietic stem cells [141]. Those differences may reflect the mitotically active and quiescent state of pluripotent stem cells versus bulge/hematopoietic stem cells, respectively. The efficient but error-prone NHEJ repair mechanism in bulge stem cells promotes their short-term survival at the cost of their long-term genomic stability. These findings highlight the notion that stem cells in general exhibit a higher efficiency in DNA damage repair, but the preferred choice of DSB repair pathways may vary among different stem cell types.

HR

Replication-induced DSBs most commonly evoke the HR machinery and the DNA helicases/nucleases for repair [142–145]. The ssDNA exposed by the initial 5′ limited end resection recruits ssDNA-binding protein, RPA, which then assembles ATR, Rad17-Rfc2-5, and the 9-1-1 complexes to trigger G2/M arrest [69–72]. Besides replicative damage, ATR can also be activated by DSBs via an ATM-

mediated pathway in a cell cycle-dependent manner [146]. The activation of ATR turns on the HR repair mechanism by recruiting RAD51. RAD51 is homologous to the bacterial RecA, and is the core HR enzyme in eukaryotes that forms the presynaptic filament by binding ssDNAs in place of RPA [147–149]. The nucleoprotein complex of ssDNA-bound RAD51 is stabilized by RAD51 paralogues, which include five members in human, that is, XRCC2, XRCC3, RAD51B/RAD51L1, RAD51C/RAD51L2, and RAD51D/RAD51L3. RAD51 serves a key role in initiating strand invasion at the homologous sequence and driving the branch migration of the Holiday junction [150]. The mechanism for RAD51 recruitment following replication stalling is not entirely clear, but may involve breast cancer 2 (BRCA2) [151], SUMOylated BLM [152], SUMOylated RPA70 [153], RAD52 [154], and X-ray repair cross-complementing proteins 2 and 3 (XRCC2 and XRCC3) [155, 156]. In mice, germ-line deletion of RAD51 results in early embryonic lethality [157]. If the end-resection process uncovers direct repeat sequences, both ssDNA ends can be annealed and repaired by a process called SSA. SSA is a RAD51-independent repair mechanism regulated by RAD52, ERCC1, and XPF. SSA repair often leads to deletions of the sequence between repeats—a completely different outcome from HR-based repair in terms of the fidelity of repair. It is therefore an undesirable choice for DSB repair in stem cells.

It is noted that HR, although accurate in its repair, operates with a very slow kinetics, which poses a challenge for fast dividing cells with a large genome size. Therefore, in fast dividing higher eukaryotic cells that primarily use HR for genome maintenance, such as in the case of mouse ES cells, the HR activity needs to be boosted so that stalled/collapsed replication forks can be restarted/repaired in a timely manner and that their genome integrity can be maintained. In many other types of higher eukaryotic cells, HR appears to play a minor role in DSB repair, as C-NHEJ repair is more efficient than HR and is active throughout the cell cycle. The exact mechanisms controlling the preferential choice of HR over C-NHEJ in mitotically active stem cells have yet to be elucidated. Generally speaking, human ES cells show higher expression levels of HR repair genes, such as RAD51 and RAD54, compared to differentiated cells [113, 133, 158]. Another way to address this issue is to find new targets that are required for stem cell self-renewal and play a role in promoting HR repair. One such candidate is a stem and cancer cell-enriched protein with a well-established function in self-renewal maintenance—nucleostemin (NS) [159–163]. NS has been shown to play indispensable roles in several fundamental biological events, including early and late embryogenesis, adult tissue regeneration, and pluripotency reprogramming [164–170]. We recently discovered its key mechanism of action in protecting proliferating cells from DNA damage during the S-phase [168, 169, 171–174], which highlights the importance of genome maintenance in self-renewal and suggests NS as a new regulatory component in the repair of replicative DNA damage [174, 175]. The role of NS in genome protection was first discovered by its ability to reduce telomeric DNA damage [171]. It was shown that NS mechanistically promotes the SUMOylation of TRF1 and the telomeric recruitment of PML-IV through interaction with SUMOylated TRF1. More recently, a role of NS in protecting against replication-induced damage on non-telomeric chromosomes was uncovered in developing stem/progenitor cells and regenerating hepatocytes [168, 169]. Our data showed that NS-knockout (NSKO)-induced DNA damage occurs independently of the p53 status or rRNA synthesis, and that NS is directly recruited to DNA damage sites and regulates the recruitment of RAD51 to stalled replication-induced DNA damage foci. Early studies suggested a link between NS and the MDM2-p53 pathway [159, 176–178]. However, it is now clear that the obligatory effect of NS loss on cell proliferation and survival occurs in the absence of p53 [165, 179–181]. Our current model states that the ability of NS to protect the integrity of telomeric and non-telomeric chromosomes during genome replication is required for the maintenance of self-renewal. It operates constitutively by the nucleoplasmic pool of NS proteins. In contrast, the MDM2-regulatory function of NS is mostly silent under normal growth conditions and becomes activated only when the nucleolar organization is disassembled under nucleolar stress conditions (Fig. 4).

Alt-NHEJ

An alternative repair pathway, Alt-NHEJ, is defined as an end-joining event that does not require Ku proteins or DNA ligase 4. This alternative repair mechanism was first observed two decades ago in C-NHEJ-deficient yeasts and mammalian cells [182–185]. It has been questioned ever since whether Alt-NHEJ is simply a by-product of persistent reactive DSB ends that are repaired by surrogates when C-NHEJ and HR are unavailable [186] or stands as an evolutionarily conserved, bona fide end-joining repair pathway [187]. A recent study showed that Alt-NHEJ can occur at approximately 10 % of the C-NHEJ efficiency in C-NHEJ-proficient as well as C-NHEJ-deficient cells [188]. Another study reported the discovery of Alt-NHEJ in *E. coli*, which lacks C-NHEJ components [189]. These findings indicate that Alt-NHEJ is a mechanistically distinct pathway in its own right that might have preceded C-NHEJ in evolution [190]. Biologically, this pathway may help save genetic information at the cost of introducing mutagenic events when more accurate mechanisms of

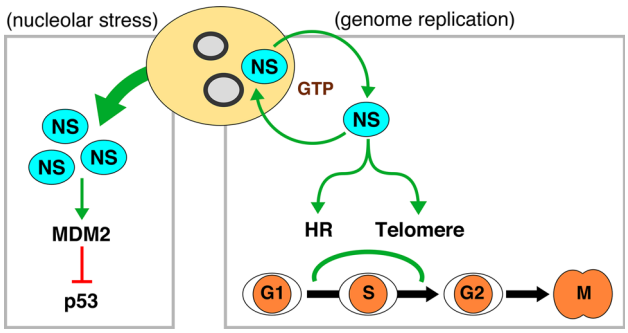

Fig. 4 NS promotes HR repair of replication-triggered DNA damage in stem cells. Our current model states that the obligatory function of NS resides in its ability to maintain the integrity of replicating genome. NS does so by promoting HR repair of DNA damage in the S-phase via a RAD51-mediated mechanism and/or a TRF1-mediated mechanism. So far, there is no evidence to indicate that localization in the nucleolus (*yellow circle*) is required for the essential activity of NS. When the nucleolus is dissembled under nucleolar stress conditions, most of the nucleolar contents, including NS, are released into the nucleoplasm in bulk. The massive increase of NS in the nucleoplasm triggers its interaction with MDM2 and thereby suppresses the p53 activity. The *green* and *red arrows* indicate an excitatory/increase or an inhibitory/decrease effect, respectively

repair are not available during, for example, mitosis [191, 192]. Pathologically, it is recognized as the major pathway responsible for chromosomal translocation [193–195]. Our current knowledge describes that some Alt-NHEJ may operate by annealing microhomology unmasked by limited end resection. Based on this reason, it is sometimes referred to as microhomology-mediated end-joining (MMEJ), although microhomology may not always be present at the repaired junction [196, 197]. Alt-NHEJ is active during the S and G2 phase, and has the propensity to introduce deletions and chromosomal rearrangement [192, 196]. To date, the molecular mechanism underlying Alt-NHEJ is not entirely clear but appears to require enzymes that perform limited end resection, end recognition, microhomology pairing, flap removal, gap filling, and ligation [198, 199]. The initial 5′-end resection step of Alt-NHEJ is carried out by MRE11 in yeasts [197, 200] and mammals [201–203], and is also mediated by CtIP [204]. The protein involved in sensing DSB ends for Alt-NHEJ is believed to be PARP1. PARP1 has been shown to promote Alt-NHEJ by competing with Ku proteins for free DSB ends [205–209]. In arabidopsis, PARP mutants display less Alt-NHEJ products [210]. Removal of non-homologous flaps may be performed by the RAD1/RAD10 endonuclease in yeasts [197, 211] or the ERCC1-XPF (ERCC4) complex in mammals [212]. Finally, gap filling and ligation may require Werner syndrome ATP-dependent helicase (WRN), DNA polymerase λ [Pol λ (lamda)], DNA ligase 3/XRCC1, and DNA ligase1 [205, 207, 213–219]. Other than these promoting factors, Alt-NHEJ may be suppressed by PARP2 and proteins involved in driving the repair decision toward C-NHEJ and possibly HR [207]. As Alt-NHEJ often leads to variable-sized interstitial deletions, inversion, chromosomal translocation, and telomere fusion [220, 221], its activity needs to be tightly controlled in stem cells.

Repairing without actual repairing: the immortal strand hypothesis

An appealing but still controversial theory of chromosome cosegregation was proposed nearly four decades ago (Fig. 5). This theory explains how long-term dividing cells (i.e. adult stem cells) at steady state minimize the consequence of replication errors or replicative damage by selective cosegregation of the parental templates or chromosomes (the immortal DNA strands) from the newly synthesized daughter chromosomes (the mortal DNA strands) [222]. This phenomenon has been described in somatic stem cells undergoing asymmetric cell division in the epidermis [223], small intestinal crypts [224], mouse mammary epithelium [225], and muscle satellite cells [226, 227]. It has also been shown in cultured cells engineered with an inducible p53 [228], as well as in neural stem cells [229]. A modified CO-FISH method was recently developed to differentially label sister chromatids with unidirectional probes to telomeric satellite DNAs [230]. Using this CO-FISH method, it was observed that apparent non-random segregation of sister chromatids occurs in a subset of colon crypt epithelial cells, which supports asymmetry of template DNA strand segregation [230]. By retaining the original DNA templates and passing the newly synthesized DNA strands down to their differentiated progeny, those stem cells are guaranteed to dodge the high frequency of replicative DNA damage literally in a repair-free manner. Some studies have begun to address the potential mechanisms underlying the selective cosegregation of parental chromosomes. In adult skeletal muscle, stem cells with long-term self-renewal express more Pax7 than cells undergoing myogenic commitment. It has been shown by the CO-FISH analysis that the Pax7-high subpopulation displays a high frequency of template DNA strand cosegregation, whereas Pax-low subpopulation separates their chromatids randomly. Some satellite cells display non-random segregation of template DNA strands and the Numb protein during growth in muscle fibers in vivo as well as in culture [226]. Cardiac progenitors also exhibit asymmetrical chromatid segregation, where Pim-1, which is a kinase that enhances cardiac repair, plays a role by increasing the asymmetrical chromatid segregation and promoting self-renewal of cardiac progenitors [231]. Conversely, there are studies that refute the immortal DNA strand theory. For example, one study showed that sister

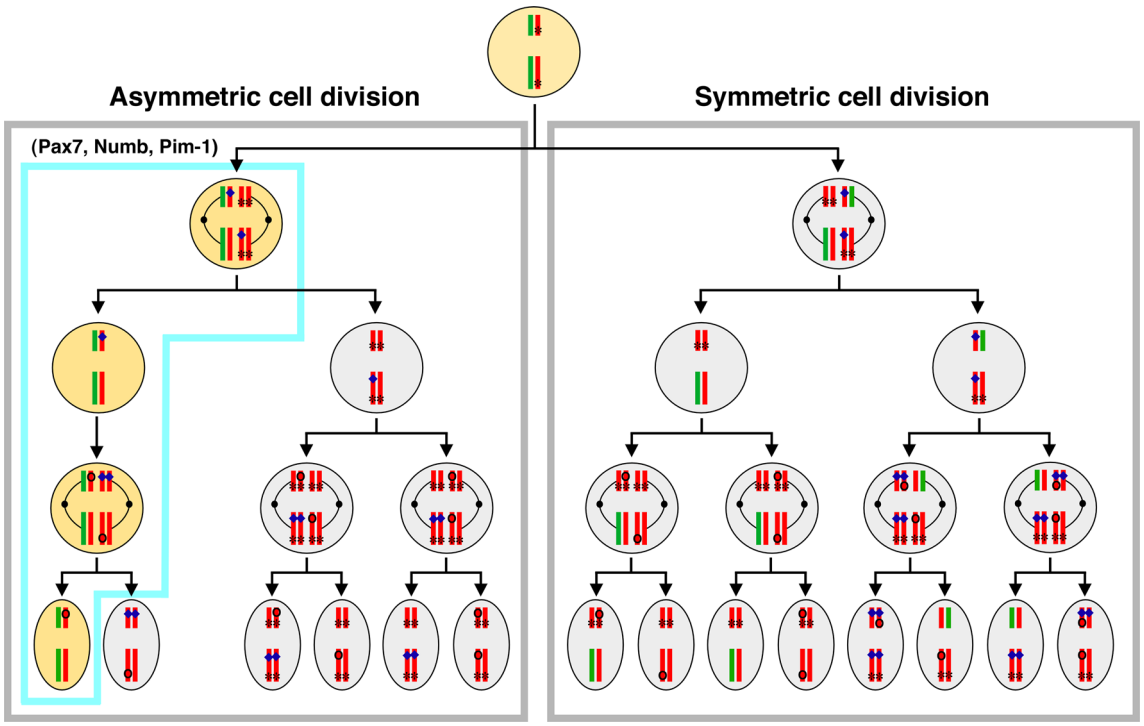

Fig. 5 The immortal strand hypothesis. Adult stem cells (*yellow*) that undergo asymmetric cell division may display a phenomenon, where the parental template DNAs (the immortal DNA strands, marked by *green*) are cosegregated into one daughter cell (i.e. stem cells) and the newly synthesized DNAs (the mortal DNA strands, marked by *red*) are passed down to the other daughter cells (i.e. progeny). For those that divide symmetrically, the template (*green*) strands are randomly segregated so that each daughter cells, no matter whether they are stem cells or differentiated cells, have equal chances to inherit the errors created during genome replication. The immortal strand theory explains why some adult stem cells accumulate less chromosomal mutations than expected, despite their long lifespan and self-renewal activity. Mutations accumulated during first to third round of genome replication are symbolized by *asterisks*, *blue diamonds*, and *open circles*, respectively

chromatids display random distribution between daughter cells in cultured lung fibroblasts and ES cells [230]. Another recent study reported that the accumulation rate of mutations in healthy stem cells of the colon, blood, head, and neck tissues are strikingly similar to those expected without the protection from the immortal strand mechanism [232]. Using the chromosome labeling approach, one group demonstrated that crypt base columnar stem cells in adult intestinal crypts segregate most of their chromosomes randomly both in intact and in regenerating epithelium [233]. Taken together, the published data seem to suggest that adult skeletal muscles and epithelial cells may reduce the long-term impact of replication-associated mutagenesis by retaining the original DNA strands in the quiescent stem cell population with self-renewal capacity. The ability of template DNA strands to cosegregate allows long-term stem cells to avoid transmitting erroneous genetic information to inherited daughter stem cells. This skill, however fascinating, may not be commandeered by all stem cells and may rely heavily on the cell division pattern (asymmetric versus symmetric cell division). In addition, it may be confounded by the methods used to detect the existence of immortal DNA strands. Finally, the immortal strand hypothesis cannot fix the problem of replication-unrelated damages that can be directly inflicted upon both chromosomal strands.

The lesser of two evils: choosing between survival with defects and death

In response to DNA damage, stem cells, like all other types of cells, recruit an evolutionarily conserved pathway, known as DNA damage response or DDR, which induces cell cycle arrest and activates DNA damage repair mechanisms [234]. The ultimate goal of the DDR pathway is to restore the damaged DNA and maintain cell survival. Under the condition when a complete reversal of genomic damage cannot be achieved and the resulting damage cannot be tolerated, those that harbor the damaged chromosomes will be eliminated by apoptosis, become senescent, undergo differentiation, or resume cycling at the risk of oncogenic transformation or mitotic catastrophe. Even though immortal DNA theory appears to be an ideal solution for tissues to eliminate damaged chromosomes without the cost of depleting the original stem cell pool,

still it remains a theory and may not be applicable to all conditions for reasons stated above. Therefore, some consequences would have to be taken under most situations. It remains an intriguing question how stem cells weigh among these various outcomes when faced with improperly repaired chromosomal damage.

Senescence is when cells stay in the G0 phase indefinitely. Senescence, along with the cell death event, ensures that the damaged chromosomes will not be passed down to other stem cells or their progenies. p53 is still the key regulator of senescence and cell death in stem cells. While disarming of the p53 guardian mechanism does allow more stem cells to survive with a defective genome, it also exposes them to higher risks of tumorigenicity [235]. Contrarily, excessive use of the p53 mechanism will eventually result in the depletion of stem cells, which may then lead to impaired tissue homeostasis, organ failure, and premature aging. This idea has been supported by studies showing that, on one hand, p53 hyperactivation is associated with bone marrow failure [236, 237], and, on the other hand, p53 deficiency promotes blood production and leukemia development at the same time [238, 239]. It has been reported that, in mice, adult hematopoietic stem cells and bulge stem cells in hair follicles are more resistant to IR exposure compared to other blood cells and epidermal cells, respectively [138, 240–242]. The relative resistance to IR in those stem cells may be explained by their higher Bcl2 expression level and shorter duration of p53 activation [138, 140]. In contrast, intestinal stem cells undergo massive cell death in response to DNA damage, which may be caused by their lower Bcl-2 expression and longer duration of p53 activation [243, 244]. Similar to intestinal stem cells, fetal hematopoietic stem cells are also more sensitive to IR exposure than their progeny cells [245]. The difference in response to DNA damage between fetal and adult hematopoietic stem cells may reflect their respective developmental natures as well as their distinctive proliferative properties (i.e. asymmetric versus symmetric cell division). Autophagy is another mechanism that promotes the survival of stem cells under genotoxic and metabolic stress conditions [246]. Autophagy is a highly conserved pathway that removes and recycles damaged organelles sequestered in autophagosomes. As we begin to understand more about the biological role of autophagy and its molecular regulation and participating molecules, now is the time to examine how this event plays into the self-renewal of various stem cells. To begin with, autophagy may contribute to the low mitochondrial mass seen in some stem cells, given its close connection to the regulation of mitochondrial activity. Readers interested in the relationship between autophagy and stem cell self-renewal and differentiation may find more information in a review article recently published [247]. Finally, differentiation is another outcome of stem cells in stress. It has been shown that oxidative stress can induce the differentiation of hematopoietic stem cells [248]. Melanocyte stem cells also undergo DNA damage-induced differentiation [249]. The differentiation of stem cells following DNA damage may be mediated by a STAT3-regulated mechanism that increases the expression of BATF [250]. Together, the current data indicate that a combination of pathways, including p53, Bcl-2 family genes, autophagy, and JAK-STAT, may cooperatively determine the survival versus apoptosis outcomes of stem cells.

Last but not least: repairing chromosomal damage at the end

Telomeres are key protectors of chromosomal integrity but prone to damage during the DNA replication process. On one hand, DNA replication shortens the telomere length. On the other hand, it may introduce breaks on the double-stranded telomere repeat region due to replication fork stalling. Therefore, maintaining telomere integrity has been a major task for all dividing cells, particularly those undergoing self-renewing proliferation. Indeed, telomere dysfunction is linked to several aging disorders and cancers [251–255]. In most cells, the telomere length is maintained primarily by the telomerase complex [256, 257]. Therefore, it should come as no surprise that male germ line and most stem cells show high levels of telomerase activity. While the discovery of the telomerase complex nicely resolves the end-replication problem, it may not represent the whole picture of telomere biology. It was noted that hematopoietic stem cells from mice overexpressing telomerase reverse transcriptase (TERT) can be serially transplanted only to the same amount of times as those isolated from wild-type mice [258]. This result indicates that the telomere length is not the sole determinant of the longevity of hematopoietic stem cells. Other factors, such as the integrity of telomeric and non-telomeric chromosomes, come into play as well. In 10–15 % of human cancers, the telomerase activity is undetectable, and the telomere length is maintained by the alternative lengthening of telomere (ALT) mechanism. Those cells, also known as ALT cells, are characterized by the hallmarks of telomere sister chromatid exchange (T-SCE), extrachromosomal telomere repeats (ECTR), heterogeneous telomere length, and ALT-associated PML bodies (APB) [259, 260]. APB is a cell biological entity defined by the overlapping of telomere foci and PML bodies. It is believed that ALT cells may use the HR mechanism for telomere elongation. While the appearance of T-SCE and ECTR may be the result of telomere HR, the biological significance of APB remains to be found. Some have postulated that APB may serve the

function of sequestering low molecular weight telomeric DNAs [261]. Others suggest that APB may be actively involved in the HR event [259, 262–265]. Interestingly, it was reported that telomere lengthening is carried out by a recombination-based, ALT-like mechanism during the early cleavage cycles after fertilization, which later transitions into the telomerase-based mechanism [266]. Reciprocally, whether the ALT state can be established from TA cells and how it is done if so happens remains unclear in a general sense. For a few selective TA cell types, e.g. HCT15 cells and T cell lymphoma, ALT can be induced by TERT inhibition or telomerase RNA component (TERC) deletion, respectively [267, 268]. One study showed that the 5′ cytosine-rich overhangs at the telomere may be linked to the HR program [269]. Other studies have identified a strong correlation between the ALT state and alpha thalassemia/mental retardation syndrome X-linked (ATRX, also known as RAD54) gene mutation in pancreatic neuroendocrine tumors, pediatric glioblastoma, and TA-ALT hybrid cells [270–272], which suggests that ATRX might be an ALT repressor.

Conclusion

Stem cells are at a higher risk of incurring DNA damage than their differentiated progeny because of their longevity in life and self-renewal requirement. The amount of damage accumulated on the genome becomes a major limiting factor that restricts their proliferative lifespan. As outlined in this review, there is good evidence to support that stem cells take care of this problem by obliterating the occurrence of DNA damage at first sight, accelerating the repair process, and selecting the less detrimental outcome (Fig. 1). Failure to do so may result in grave consequences, including organ failure, premature aging, and/or cancer formation. Such is the case with Cockayne syndrome, Werner syndrome, ataxia telangiectasia, xeroderma pigmentosum, trichothiodystrophy, and Hutchinson-Gilford progeria. While stem cells in general may be equipped with a heightened defense against genomic stress, they do not always do it in the same way. Mitotically active stem cells tend to use HR for damage repair and select apoptosis as the outcome in exchange for long-term genomic stability, whereas quiescent stem cells tend to do the opposite. It is my hope that a review on this subject may have a measurable impact on our understanding of the biology underlying tissue homeostasis, premature aging, and tumor formation through stating the current state of knowledge on how different types of stem cells maintain their genome integrity while undergoing self-renewal throughout life and building a conceptual framework to catalyze future research.

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Compliance with ethical standards

Conflict of interest The author declares no conflict of interest.

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