CellPress

Molecular Cell

Technology review

CRISPR-based genome editing through the lens of DNA repair

Tarun S. Nambiar,[1] Lou Baudrier,[2] Pierre Billon,[2,*] and Alberto Ciccia[1,3,*]
[1]Department of Genetics and Development, Herbert Irving Comprehensive Cancer Center, Columbia University Irving Medical Center, New York, NY 10032, USA
[2]Department of Biochemistry and Molecular Biology, Robson DNA Science Centre, Arnie Charbonneau Cancer Institute, Cumming School of Medicine, University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta T2N 4N1, Canada
[3]Lead contact
*Correspondence: pierre.billon@ucalgary.ca (P.B.), ac3685@cumc.columbia.edu (A.C.)
https://doi.org/10.1016/j.molcel.2021.12.026

SUMMARY

Genome editing technologies operate by inducing site-specific DNA perturbations that are resolved by cellular DNA repair pathways. Products of genome editors include DNA breaks generated by CRISPR-associated nucleases, base modifications induced by base editors, DNA flaps created by prime editors, and integration intermediates formed by site-specific recombinases and transposases associated with CRISPR systems. Here, we discuss the cellular processes that repair CRISPR-generated DNA lesions and describe strategies to obtain desirable genomic changes through modulation of DNA repair pathways. Advances in our understanding of the DNA repair circuitry, in conjunction with the rapid development of innovative genome editing technologies, promise to greatly enhance our ability to improve food production, combat environmental pollution, develop cell-based therapies, and cure genetic and infectious diseases.

INTRODUCTION

Genome stability is constantly threatened by DNA lesions induced by endogenous and environmental DNA damaging agents (Ciccia and Elledge, 2010). Living organisms have developed a network of cellular pathways, collectively referred to as the DNA damage response (DDR), that maintains genome stability in response to DNA damage. Highly conserved cellular DNA repair processes include double-strand break (DSB) repair, single-strand break (SSB) repair, mismatch repair (MMR), and base and nucleotide excision repair (BER and NER, respectively). Each of these pathways is specialized in the detection and resolution of distinct types of DNA lesions. DNA repair pathways are extensively interconnected, as highlighted by recent genetic investigation of the DDR network in human cells (Olivieri et al., 2020).

Genome editing tools, such as CRISPR-based technologies (Doudna, 2020; Knott and Doudna, 2018; Liu et al., 2022), generate targeted DNA lesions that are resolved through a complex interplay of DNA repair pathways (Chen et al., 2021b; Hussmann et al., 2021; Koblan et al., 2021a). Site-specific DNA nucleases introduce DSBs, which can undergo error-prone or error-free repair, resulting in DNA mutagenesis or the insertion of desired changes, respectively, at a locus of interest (Figure 1) (Jasin and Haber, 2016). Inactivated DNA nucleases fused to or co-expressed with DNA modifying enzymes generate a variety of DNA lesions and structures, including SSBs, modified bases, abasic sites, mismatched nucleotides, DNA flaps, and integration intermediates, whose resolution can lead to desired base substitutions and deletions or insertions of interest (Figure 1) (Anzalone et al., 2020). These DSB-free genome editors mitigate the cellular risks associated with the induction of genomic DSBs by site-specific DNA nucleases. Based on their distinct features, genome editors display different strengths and weaknesses, as highlighted in Anzalone et al. (2020), Hayward and Ciccia (2021), and Newby and Liu (2021). In this review, we discuss genome editing technologies with an emphasis on the distinct types of DNA lesions induced by genome editors. In addition, we highlight the main players of the DNA repair pathways implicated in the resolution of those DNA lesions and discuss applications and limitations of genome editing technologies.

DSB-BASED GENOME EDITING

The induction of site-specific DSBs to modify the genome is the cornerstone of genome editing technologies (Jasin and Haber, 2016). Indeed, the discovery that genome editing is stimulated by DSB formation induced by meganucleases (e.g., I-SceI) spearheaded the search and development of programmable nucleases (Jasin and Haber, 2016; Rouet et al., 1994). The generation of site-specific genome editing tools was initially enabled by combining the non-specific FokI nuclease domain to sequence-specific DNA binding proteins, such as zinc-finger binding domains and TAL effectors (Bibikova et al., 2003; Boch et al., 2009; Kim and Kim, 2014; Moscou and Bogdanove, 2009; Urnov et al., 2005). More recently, the emergence of RNA-guided genome editing systems that employ CRISPR-Cas proteins has accelerated the use of DSB-based genome editing technologies.

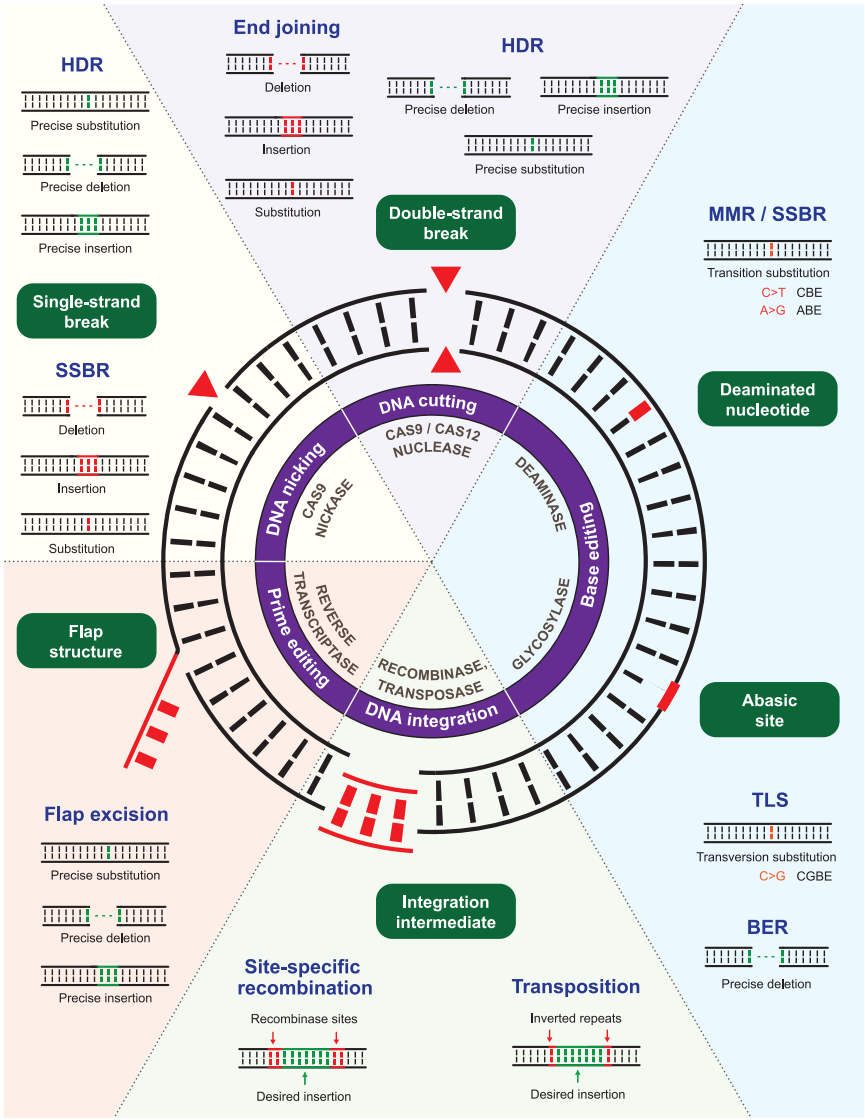

Figure 1. Site-specific DNA perturbations induced by CRISPR-based genome editing technologies and DNA repair processes that resolve them

Schematic of the five major CRISPR-based genome editing technologies and six site-specific DNA lesions/structures generated by them. Enzymatic activities of genome editors generating the depicted DNA lesions/structures, DNA repair processes involved in their resolution, and outcomes of the repair events are also illustrated. BER, base excision repair; HDR, homology-directed repair; MMR, mismatch repair; SSBR, single-strand break repair; TLS, translesion synthesis.

DSB induction by programmable nucleases derived from CRISPR-Cas systems

CRISPR–Cas is a prokaryotic adaptive defense system that targets and eliminates invading nucleic acids to circumvent predation by viruses and other mobile genetic elements (Koonin et al., 2017). Specificity for DNA/RNA targets is achieved through small non-coding RNAs, known as guide RNAs (gRNAs), which constitute the cellular memory of past infections (Gasiunas et al., 2012; Jinek et al., 2012; Schneider, 2020; Shivram et al., 2021). In the case of a secondary encounter with the same infectious agent, gRNAs form base pairs with the intruder's genome, leading to nucleic acid cleavage by Cas proteins, followed by its elimination (Garneau et al., 2010; Nussenzweig and Marraffini, 2020).

CRISPR systems are classified into two classes based on the architecture of the effector complexes (Makarova et al., 2020). In Class 1 CRISPR systems, which are the most abundant in prokaryotes, DNA interference is mediated by a multi-protein complex, known as CRISPR-associated complex for antiviral defense (Cascade), through its Cas3 helicase/nuclease subunit (Brouns et al., 2008). The Cascade complex has been harnessed for genome engineering applications to induce site-specific deletions, introduce new genomic sequences, and regulate gene transcription (Cameron et al., 2019; Chen et al., 2020; Csörgő et al., 2020; Pickar-Oliver and Gersbach, 2019). Class 2 CRISPR systems are composed of single-subunit effector nucleases with distinct, and highly diverse, functional properties (Makarova et al., 2020; Stella et al., 2017). For example, different Class 2 effectors display nuclease activities directed against ssDNA (Harrington et al., 2018), dsDNA (Gasiunas et al., 2012; Jinek et al., 2012; Karvelis et al., 2020; Liu et al., 2019; Pausch et al., 2020), or RNA (Abudayyeh et al., 2016, 2017; Cox et al., 2017). Within this class, the type II and V systems are based on the RNA-guided DNA endonuclease families of Cas9 and Cas12. Cas9 employs two distinct nuclease domains to achieve DSB formation, an HNH domain that cleaves the gRNA-targeted strand and a RuvC domain that cleaves the non-targeted DNA strand. Inactivation of either of the nuclease domains generates a nickase Cas9 (nCas9) mutant (Gasiunas et al., 2012; Jinek et al., 2012), while inactivation of both nuclease domains generates a catalytically dead Cas9 (dCas9) (Qi et al., 2013). Cas9 nickases and catalytically dead variants constitute the building blocks of DSB-free genome editors. The Cas12 family is characterized by multiple variants with unique features (Makarova et al., 2020; Yan et al., 2019). Cas12a is the type V nuclease most widely utilized for genome editing applications (Zetsche et al., 2015). Additional Cas12 variants include Cas12b (Strecker et al., 2019a; Teng et al., 2018), Cas12d (also known as CasY) (Burstein et al., 2017), Cas12e (also known as CasX) (Burstein et al., 2017; Liu et al., 2019), Cas12f (also known as Cas14) (Harrington et al., 2018), Cas12j (also known as CasΦ) (Pausch et al., 2020), Cas12h, Cas12i, and Cas12c (Yan et al., 2019). A subset of

Cas12 enzymes (e.g., Cas12a, Cas12i) process their own CRISPR gRNA array without accessory factors (Fonfara et al., 2016; Liao and Beisel, 2021; Yan et al., 2019), thus enabling multiplexed genome editing and gene regulation (Campa et al., 2019; Zetsche et al., 2017). Certain Cas12 nucleases (e.g., Cas14, CasY, CasX, CasΦ) are characterized by a small size, which could facilitate *in vivo* genome editing applications using viral vectors with limited packaging size (Kim et al., 2021a; Wang et al., 2020a; Xu et al., 2021b). Recently, by reconstructing the evolution of the Cas9 and Cas12 endonucleases, a family of highly abundant transposon-encoded RNA-guided systems, called OMEGA, was discovered in both prokaryotes and eukaryotic cells (Altae-Tran et al., 2021; Karvelis et al., 2021). Although these widespread programmable nucleases, including IscB, IsrB and TnpB, hold great promise for genome targeting and editing, their activities and biological relevance are still not fully characterized.

Cellular mechanisms that repair DSBs introduced by programmable nucleases

DSBs introduced by DNA nucleases are primarily repaired by end joining mechanisms, which are often error-prone, or homology-directed repair (HDR), which is typically error-free (Figure 2) (Scully et al., 2019). End joining involves the direct ligation of the two DSB ends, with or without end processing. End processing can lead to the addition or removal of nucleotides at the DSB ends, resulting in insertion or deletion (indel) mutations. End joining can occur through non-homologous end joining (NHEJ) or microhomology-mediated end joining (MMEJ) (Chang et al., 2017). Distinct from end joining, HDR generally utilizes homologous sequences located on sister chromatids or, less commonly, homologous chromosomes to repair DSBs (Chen et al., 2018b). The HDR machinery can also repair DSBs using exogenous DNA donor templates, enabling the insertion of a wide array of desired modifications at defined genomic loci for precision genome editing applications.

End-joining-mediated genome editing

Non-Homologous End Joining. NHEJ, the predominant DSB repair pathway in mammalian systems, takes place with rapid kinetics throughout the cell cycle (Figure 2, steps 1 and 2) (Hustedt and Durocher, 2016; Mao et al., 2008). After a DSB has formed, the broken ends are quickly bound and protected by the Ku70–Ku80 (Ku) heterodimer (Chang et al., 2017; Mimitou and Symington, 2010). Ku then recruits and activates the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), whose kinase activity is essential for NHEJ (Frit et al., 2019). Ku also recruits the NHEJ factors XRCC4, XLF, and DNA ligase IV (LIG4) (Zhao et al., 2019). NHEJ factors initially form a long-range synaptic complex that tethers the two DSB ends (Chen et al., 2021c; Graham et al., 2016; Stinson and Loparo, 2021). DNA-PKcs autophosphorylation leads to its dissociation from DNA and the transition to a short-range synaptic complex in which the DSB ends are closely aligned to enable end processing and subsequent ligation by LIG4 (Chen et al., 2021c; Graham et al., 2016; Stinson et al., 2020). The short-range synaptic complex ensures that the DNA ends undergo ligation as soon as they become compatible, thereby restricting DNA end processing and minimizing mutagenesis (Stinson et al., 2020).

Microhomology-Mediated End Joining. MMEJ was originally discovered in NHEJ-deficient cells as a backup pathway for DSB repair (Sfeir and Symington, 2015). Similar to NHEJ, MMEJ joins the broken DSB ends without the need for homologous templates (Figure 2, steps 3–5). However, unlike NHEJ, MMEJ requires initial DSB end resection and functions independently of Ku and LIG4 (Sfeir and Symington, 2015). Moreover, MMEJ operates on short homologous sequences (“microhomologies”), ranging between ~5 and 25 bp in mammalian cells, that lie close to both DSB ends (McVey and Lee, 2008). MMEJ is initiated by 5′ to 3′ resection of the DSB ends by the MRE11–RAD50–NBS1 (MRN) nuclease complex together with its stimulatory factor CtIP (Anand et al., 2016; Reginato and Cejka, 2020; Truong et al., 2013). MRN-CtIP-mediated resection of the DSB ends has two important consequences. First, it promotes the removal of Ku and DNA-PKcs, thereby inhibiting NHEJ-mediated repair (Chanut et al., 2016; Deshpande et al., 2020; Reginato et al., 2017). Second, by exposing potential ssDNA microhomologies, it allows the annealing of the broken ends, followed by end trimming and extension by the DNA polymerase Polθ (Brambati et al., 2020; Ramsden et al., 2021; Zahn et al., 2021). The FEN1 endonuclease can then remove the resulting 5′ flaps, and DNA ligase I or III ligates the remaining nicks to complete DSB repair (Brambati et al., 2020; Ramsden et al., 2021). Recent studies have also implicated RAD17 and the associated RAD9-HUS1-RAD1 (9-1-1) complex in MMEJ (Hussmann et al., 2021), albeit the role of these factors remains to be elucidated. Although MMEJ and NHEJ protect cells against the severe threat posed by DSBs, when they operate inaccurately they can cause genomic rearrangements, such as chromosomal translocations, intra- and inter-chromosomal deletions and insertions, and end-to-end chromosomal fusions (Dahiya et al., 2021; Ramsden and Nussenzweig, 2021).

DNA Repair Outcomes Induced by End Joining during Genome Editing. During genome editing, reconstitution of the target sequence by error-free NHEJ-mediated repair (i.e., in the absence of end processing) of Cas9-induced DSBs results in repeated Cas9 cleavage cycles until mutagenic NHEJ events block gRNA target recognition (Brinkman et al., 2018). Therefore, Cas9-mediated genome editing ultimately enriches for mutagenic outcomes induced by templated or non-templated nucleotide insertions and deletions (Figure 2, bottom left) (Allen et al., 2019; Chakrabarti et al., 2019; Chen et al., 2019; Leenay et al., 2019; Shen et al., 2018; Shou et al., 2018; van Overbeek et al., 2016). In line with these observations, the DNA polymerase Polλ was recently shown to promote mutagenic fill-in of Cas9-mediated DSBs, and this activity is inhibited by NHEJ factors (Hussmann et al., 2021). In the presence of microhomologies flanking Cas9-induced DSBs, MMEJ-mediated DSB repair can result in the formation of Polθ-dependent deletions (Taheri-Ghahfarokhi et al., 2018). The frequency of MMEJ-induced deletions correlates positively with GC base content, microhomology length, and proximity to the DSB site (Allen et al., 2019; Chakrabarti et al., 2019; Shen et al., 2018). While microhomologies with mismatches can also generate deletions, the presence of mismatches reduces the frequency of these events (Allen et al., 2019).

Recent studies have shown that the mutational profiles generated by end joining events at Cas9-induced DSBs are largely

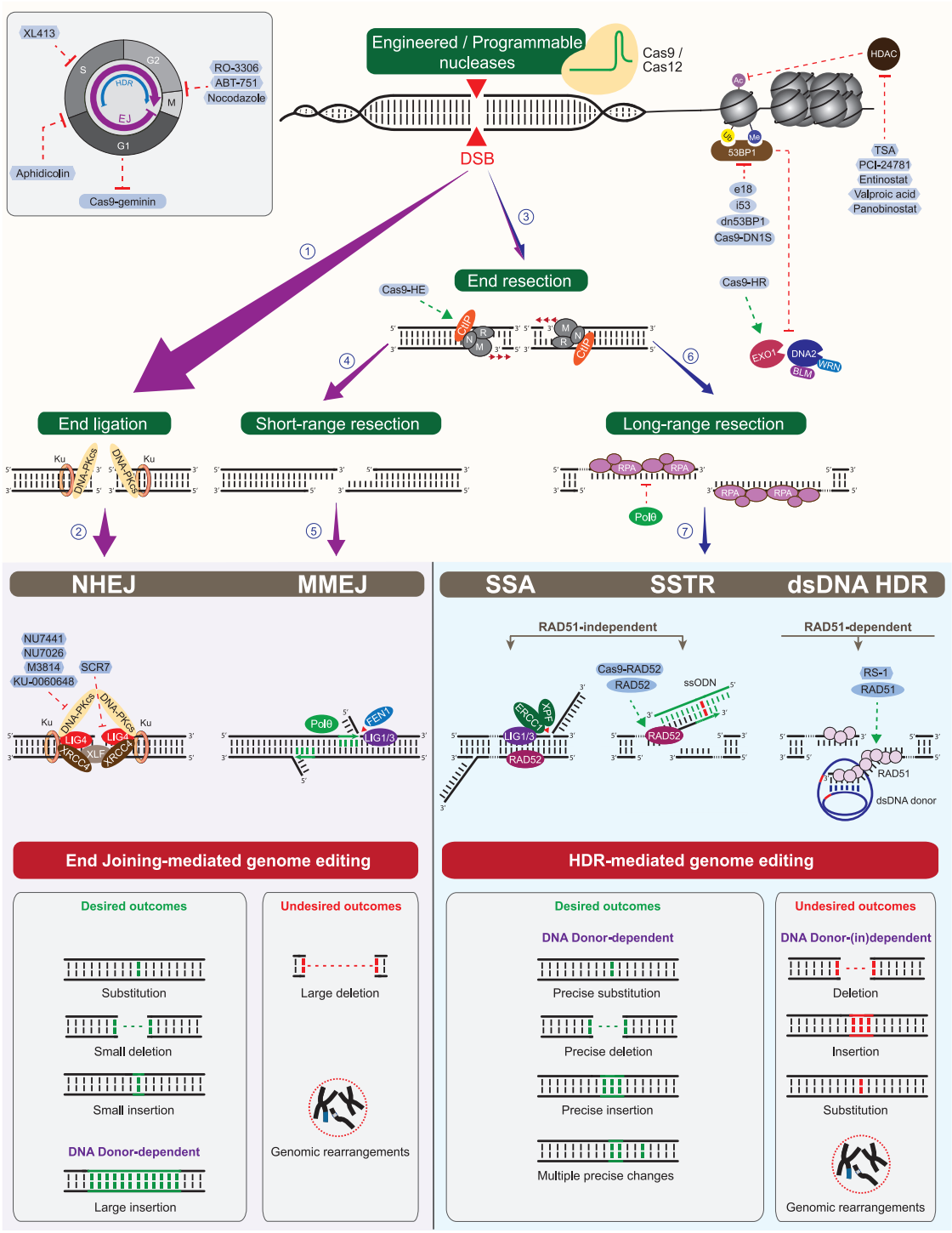

Figure 2. Repair of DSBs induced by site-specific DNA nucleases and strategies to stimulate precision genome editing

DNA double-strand breaks generated by engineered or programmable nucleases can either be repaired by end joining (left) or HDR mechanisms (right). While end joining can occur throughout the cell cycle, HDR is confined to the S and G2 phases of the cell cycle when a sister chromatid is available for recombination (top left). The choice between end joining and HDR is regulated by DNA end resection. DSB ends that require minimal or no end processing can be ligated by NHEJ through the activities of Ku, DNAPKcs, XLF, XRCC4, and LIG4 (1 and 2). Short-range end resection catalyzed by MRE11-RAD50-NBS1 (MRN) in complex with CtIP can expose regions of microhomology, which can undergo annealing and end joining by MMEJ (3–5). MMEJ is mediated by Polθ, which extends the annealed ends, followed by the removal of 5′ flaps by FEN1 and DNA ligation by LIG1/3 (5). End-joining-mediated genome editing creates substitutions and small indel mutations and can also generate large insertions in the presence of linearized dsDNA donors (bottom left, in green). End joining events can also result in the formation of large deletions and chromosomal rearrangements (bottom left, in red). Long-range resection is catalyzed by EXO1 or by DNA2 in complex with BLM

(legend continued on next page)

reproducible and depend on the sequence context of the Cas9-targeted site (Allen et al., 2019; Chakrabarti et al., 2019; Chen et al., 2019; Leenay et al., 2019; Shen et al., 2018; Shou et al., 2018; van Overbeek et al., 2016). The majority of the reproducible mutations at Cas9-induced DSBs correspond to NHEJ-dependent single base insertions and small deletions and MMEJ-mediated deletions (Allen et al., 2019; Chakrabarti et al., 2019; Chen et al., 2019; Shen et al., 2018; van Overbeek et al., 2016). Single base insertions are suppressed by the kinase activity of ATM (Bermudez-Cabrera et al., 2021). Together, the above observations led to the development of machine learning models (i.e., inDelphi, FORECasT, SPROUT, Lindel) that can predict the main mutational signatures resulting from end-joining-mediated repair of Cas9-induced DSBs (Allen et al., 2019; Chen et al., 2019; Leenay et al., 2019; Shen et al., 2018).

Applications of End-Joining-Mediated Genome Editing. End-joining-mediated DSB repair is employed in numerous genome editing applications (Pickar-Oliver and Gersbach, 2019). Given its mutagenic nature, end joining is often harnessed to disrupt the functionality of coding and non-coding elements. End-joining-mediated gene disruption has been used to study the function of genes in cellular and animal models, including previously genetically intractable systems, such as monkey embryos (Niu et al., 2014), or newly established genetic models, like the killifish (Harel et al., 2016). Additionally, high-throughput screening technologies with gRNA libraries that induce loss-of-function perturbations have been used to annotate functional genetic elements and discover new cancer dependencies (Shalem et al., 2015). Interestingly, the generation of multiple site-specific DSBs has also enabled the modeling of chromosomal deletions and translocations frequently associated with human cancer (Brunet and Jasin, 2018). In addition, both NHEJ and MMEJ have been exploited to insert new sequences into the genome (Figure 2, bottom left) (Nakade et al., 2014; Schmid-Burgk et al., 2016; Suzuki and Izpisua Belmonte, 2018; Suzuki et al., 2016). End-joining-based approaches enable the efficient insertion of DNA payloads in biological systems not proficient in HDR-mediated DSB repair, such as non-dividing cells (e.g., neuronal cells) and certain human organoid models (Artegiani et al., 2020; Nami et al., 2018; Suzuki et al., 2016).

End-joining-mediated gene disruption has been successfully employed to revert the effects induced by pathogenic mutations in pre-clinical and clinical settings. This approach has shown promise for the treatment of sickle cell disease and β-thalassemia, with multiple *ex vivo* strategies developed during clinical trials to inactivate *BCL11A* in hematopoietic stem and progenitor cells (Ferrari et al., 2021; Frangoul et al., 2021; Mullard, 2020). Clinical studies have also shown the potential of end-joining-dependent approaches for the *in vivo* disruption of transthyretin in patients with transthyretin amyloidosis (Gillmore et al., 2021). Moreover, recent studies have highlighted the feasibility of end-joining-mediated strategies for disrupting T cell receptors and the PD-1 immune checkpoint regulator in T cells of patients with refractory cancer (Ellis et al., 2021; Stadtmauer et al., 2020). In addition, strategies that rely on the joining of two paired Cas9-induced DSBs have been utilized to excise mutant exons of the dystrophin gene and restore its open reading frame in cells from patients with Duchenne muscular dystrophy (DMD) and DMD animal models (Amoasii et al., 2018; Amoasii et al., 2017; Choi and Koo, 2021; Min et al., 2019). Similar dual DSB-based strategies developed for excising an aberrant splicing donor site in the *CEP290* gene are currently being used in clinical trials for *in vivo* correction of the *CEP290* splicing defects that underlie Leber congenital amaurosis (Maeder et al., 2019; Quinn et al., 2021). Related approaches have also been employed to delete expanded trinucleotide repeats in cell lines derived from patients with Huntington's disease (HD), fragile X syndrome, and myotonic dystrophy type 1 (DM1) and in animal models of HD, DM1, and Friedrich's ataxia (Monteys et al., 2017; Mosbach et al., 2019; Ouellet et al., 2017; Provenzano et al., 2017; Shin et al., 2016; van Agtmaal et al., 2017; Xie et al., 2016). Finally, approaches that take advantage of the predictable mutation outcome of MMEJ-mediated DSB repair have been deployed to correct pathogenic microduplications in cells from patients with limb-girdle muscular dystrophy and Hermansky–Pudlak syndrome (Iyer et al., 2019; Shen et al., 2018). In this regard, the use of machine-learning-based algorithms that predict the mutational outcome of end joining events (Allen et al., 2019; Chen et al., 2019; Leenay et al., 2019; Shen et al., 2018) may further facilitate clinical applications of end-joining-mediated genome editing.

Donor-dependent homology-directed repair

HDR promotes the repair of DSBs using endogenous or exogenous homologous DNA templates (Gallagher and Haber, 2018; Jasin and Haber, 2016). Although HDR is a largely error-free repair mechanism, if not properly regulated, it can cause genomic rearrangements and thus result in genomic instability (Al-Zain and Symington, 2021). HDR requires extensive 5′ to 3′ resection of DNA ends, which is initiated by the MRN-CtIP complex and then extended by the exonuclease EXO1 or by the helicase-nuclease DNA2 in complex with the BLM or WRN helicases (Cejka and Symington, 2021). End resection results in the generation of 3′ ssDNA tails that promote the search of homologous sequences to be used as templates for repair (Figure 2, steps 6 and 7). In genome editing, different forms of exogenous donor templates are used to repair site-specific DSBs, including

or WRN and is inhibited by 53BP1 bound to histones with H4K20me2 (me) and H2AK15ub (ub) marks (top right). Long-range resection results in the generation of 3′ ssDNA tails that initiate HDR events (6 and 7). Annealing of homologous sequences by RAD52, followed by excision of 3′ flaps by XPF-ERCC1 and DNA ligation by LIG1/3 promotes the joining of DSB ends by SSA. SSA causes deletions and potential genomic rearrangements (bottom right, in red). In the presence of ssODNs or dsDNA donors, SSTR or dsDNA donor-dependent HDR (dsDNA HDR) promote the generation of precise DNA substitutions, deletions, insertions, and complex mutations (bottom right, in green). While SSTR is mediated by RAD52-dependent annealing of 3′ ssDNA tails to DNA donors, followed by templated DNA synthesis, dsDNA HDR is catalyzed by the recombinase RAD51, which promotes the invasion of 3′ ssDNA tails into homologous sequences of DNA donors to initiate DNA synthesis. Precision genome editing can be stimulated by end joining inhibitors or activators of end resection and HDR. Enhanced HDR can also be obtained by alteration of the cell cycle (top left) and chromatin structure (top right), modulation of the activity of DNA repair factors, expression of engineered DNA repair variants, fusion of DNA repair proteins to Cas nucleases, and modification of DNA donor molecules. HDR modulators are highlighted in blue (see also Table 1). While largely error-free, SSTR and dsDNA HDR can also cause deletions, insertions, point mutations, and genomic rearrangements when conducted inaccurately (bottom right, in red). These events can occur in a manner dependent or independent from DNA donors.

double-strand DNA (dsDNA) donors, short or long single-strand oligodeoxynucleotides (ssODN), and chromatinized templates (Cruz-Becerra and Kadonaga, 2020; Li et al., 2019; Yeh et al., 2019). The repair of DSBs using dsDNA and ssODN donors occurs through RAD51-dependent or -independent mechanisms, respectively, as discussed below.

RAD51-Dependent HDR. The RAD51 recombinase mediates homology search and strand exchange between broken DNA strands and dsDNA repair templates (Figure 2, step 7). Following RAD51-mediated strand invasion and formation of a displacement loop (D-loop) structure, the invading strand is thought to be elongated by the DNA polymerase δ using the homologous sequence as a template (McVey et al., 2016). DNA synthesis can then be followed by the dissociation of the invading DNA strand and its reannealing to the second DSB end through a process known as synthesis-dependent strand annealing (SDSA) (McVey et al., 2016). Alternatively, if the second DSB end is captured by the D-loop structure, double Holliday junctions can form, which then undergo either dissolution by the BLM-TOPO III complex or resolution by Holliday junction resolvases (Matos and West, 2014). Although it remains unclear how the choice between SDSA and double Holliday junction formation is regulated, both pathways have been implicated in repairing DSBs using dsDNA donors (Kan et al., 2014). In the absence of second end capture, the repair of nuclease-mediated DSBs can occur through break-induced replication (BIR), a process that entails extensive DNA synthesis carried out at migrating D-loop structures (Kramara et al., 2018; Llorente et al., 2008). BIR has been shown to cause DNA mutagenesis and chromosomal rearrangements (Malkova and Haber, 2012). Interestingly, recent studies have identified mechanisms that promote second end capture and D-loop dissociation to restrict mutagenic BIR (Pham et al., 2021).

RAD51-Independent HDR. RAD51-independent HDR pathways include single-strand annealing (SSA) and single-stranded template repair (SSTR) (Figure 2, step 7) (Gallagher and Haber, 2018). SSA shares similarities with MMEJ in its ability to promote the joining of DSB ends with partial or complete sequence homology. Unlike MMEJ, however, SSA utilizes long flanking sequences of homology, which can span several hundred nucleotides in mammalian cells (Bhargava et al., 2016). SSA is mediated by the HDR protein RAD52, which binds the resected ssDNA ends and anneals regions of homology, resulting in the deletion of intervening sequences (Bhargava et al., 2016).

SSTR promotes the repair of DSBs using ssODN templates (Gallagher and Haber, 2018; Storici et al., 2006). Given its higher efficiency and fidelity relative to HDR processes that use dsDNA templates and the facile access to synthesized and chemically modified ssODNs, SSTR is the most frequently utilized HDR mechanism for genome editing (Richardson et al., 2016; Yeh et al., 2019). SSTR shares similarities with multiple HDR pathways. Like SDSA, SSTR is thought to be stimulated by DSB resection (Canny et al., 2018; Gallagher and Haber, 2018; Nambiar et al., 2019), and both HDR processes involve unidirectional conversion tracts due to strand pairing and extension of one of the DSB ends (Gallagher and Haber, 2018; Kan et al., 2017; Paix et al., 2017). However, unlike SDSA, SSTR functions independently of RAD51 (Bothmer et al., 2017; Richardson et al., 2018). Instead, SSTR requires RAD52, similar to SSA, and reminiscent of RNA-templated HDR (Gallagher et al., 2020; Keskin et al., 2014; Mazina et al., 2017; Storici et al., 2006). In addition to being used as templates for HDR events, ssODNs can also directly integrate into homologous genomic sites at Cas9-induced breaks through mechanisms that remain to be fully established (Kan et al., 2017). Besides RAD52, the Fanconi anemia pathway has also been proposed to be involved in SSTR and dsDNA donor-dependent HDR through unresolved mechanisms (Richardson et al., 2018; Wienert et al., 2020). Altogether, several important aspects of the molecular mechanisms underlying SSTR remain to be elucidated, including the involvement of DSB resection machineries and the processes that mediate strand invasion, D-loop extension, and second end capture.

Applications of HDR-Mediated Genome Editing. The ability of HDR to install any desired nucleotide change into the genome makes it an attractive pathway for disease modeling. HDR-based approaches also hold great promise for clinical management of inherited and acquired human diseases (Doudna, 2020). For example, a pig model of HD was recently developed by replacing exon 1 of the pig *HTT* gene with the corresponding exon of the human *HTT* gene containing a 150-CAG repeat, enabling the recapitulation in mammalian models of the selective neurodegeneration induced by CAG triplet expansion in HD patients (Yan et al., 2018). The clinical potential of CRISPR-mediated HDR is also highlighted by the enhanced antitumor activity exhibited by T cells with a chimeric antigen receptor inserted by HDR at the T cell receptor locus (Eyquem et al., 2017). Pooled knock-in HDR-based strategies to insert large DNA cargos into the T cell receptor locus of primary human T cells have enabled the identification of gene constructs that improve T cell-mediated antitumor activity (Roth et al., 2020). Furthermore, saturation mutagenesis experiments that used HDR to install all possible substitution mutations into genomic regions of interest in haploid cells have allowed the interrogation of clinically actionable genes at a single-base level (Findlay et al., 2014, 2018). Despite the success of these studies, the low frequency of HDR events relative to concomitant mutations induced at the targeted loci by error-prone end-joining-mediated repair has limited the widespread adoption of high-throughput knock-in screens using HDR. HDR-based approaches have also been employed for endogenous gene tagging with genetic markers, enabling the detection of the subcellular localization of proteins, isolation of native protein complexes, and temporal regulation of protein function (Chen et al., 2018a; Cho et al., 2021; Dalvai et al., 2015; Leonetti et al., 2016; Miyaoka et al., 2014; Natsume et al., 2016). Finally, HDR-mediated strategies have been used to promote gene drive, a process that stimulates non-Mendelian inheritance of genetic elements, to potentially eradicate harmful species causing vector-borne diseases (Champer et al., 2016).

DDR modulating strategies to promote HDR-based precision genome editing

One of the major determinants of DSB repair pathway choice is DNA end resection. As discussed above, DNA end resection promotes DSB repair by MMEJ and HDR while generating DSB ends not suitable for NHEJ-mediated repair (Symington and Gautier, 2011). In mammalian cells, DNA end resection

operates with slower kinetics than NHEJ. It is regulated by the cell cycle and chromatin environment, ensuring that HDR occurs in the S and G2 phases when an appropriate repair template (i.e., a sister chromatid) is present (Figure 2, top left) (Hustedt and Durocher, 2016). Ultimately, DSB repair pathway choice dictates genome editing outcomes, and competition between alternative DSB repair pathways can generate mosaicism among edited alleles. Consequently, strategies to regulate DSB repair pathway choice by either stimulating or counteracting specific DSB repair pathways enable more efficient and precise genome editing.

Given the fast-acting nature of NHEJ, inhibiting this pathway is an attractive strategy for precision genome editing (Figure 2, step 2). In this regard, efforts to inhibit the core NHEJ factors have produced mixed results in human cells. For example, pharmacological inhibition of Ku70/80 marginally stimulates HDR (Table 1) (Riesenberg and Maricic, 2018). LIG4 inhibition by the small-molecule SCR7 or viral proteins that induce its proteasomal degradation are reported to increase HDR in certain human and mouse cells, although this effect was more limited in other cellular contexts (Chu et al., 2015; Greco et al., 2016; Gutschner et al., 2016; Hu et al., 2018; Maruyama et al., 2015; Pinder et al., 2015; Robert et al., 2015b; Song et al., 2016; Srivastava et al., 2012; Yang et al., 2016; Zhang et al., 2017). Such variability in effect might be partially due to the usage of different chemical derivatives of SCR7 (Yeh et al., 2019). Similarly, small-molecule inhibition of DNA-PKcs with NU7441, NU7026, and M3814 has also been reported to increase HDR (Riesenberg et al., 2019; Robert et al., 2015; Suzuki et al., 2016). Dual inactivation of both MMEJ and NHEJ also holds the promise of synergistically enhancing HDR. For example, combinatorial inactivation of NHEJ and MMEJ proteins, such as Ku70/80 and Polθ, prevents off-target integration of dsDNA donors (Zelensky et al., 2017). Given that inactivation of end joining is associated with exacerbation of sensitivity to various genotoxic agents and increased carcinogenesis (Davis and Chen, 2013; Ramsden et al., 2021), the impact of these HDR-modulating treatments on genomic integrity and cell viability needs to be carefully investigated.

Because of the central role of DSB resection in the choice between end joining and HDR, stimulation of DSB resection has shown great promise in promoting HDR. TP53-binding protein 1 (53BP1), a key inhibitor of end resection, is recruited to chromatin through the binding of its Tudor and UDR domains to histone H4 dimethylated at Lys20 (H4K20me2) and histone H2A monoubiquitylated at Lys15 (H2AK15ub), respectively (Figure 2, top right) (Botuyan et al., 2006; Fradet-Turcotte et al., 2013). Multiple strategies have been developed to stimulate HDR by inhibiting 53BP1 function (Table 1). Expression of an engineered ubiquitin variant that binds the Tudor domain of 53BP1 (i53, inhibitor of 53BP1) has been shown to prevent 53BP1 binding to chromatin and stimulate HDR (Canny et al., 2018). Similarly, a dominant-negative 53BP1 (dn53BP1) and an engineered variant of RAD18 (e18) stimulate HDR by competing with 53BP1 for binding H2AK15ub through their ubiquitin binding domains, thereby occluding 53BP1 from DSBs (Nambiar et al., 2019; Paulsen et al., 2017). Fusing Cas9 to dn53BP1 or e18 (Jayavaradhan et al., 2019; Richardson et al., 2020) also stimulates HDR with potentially improved specificity, given the targeted inhibition of 53BP1 function at Cas9-induced DSBs. Notably, some of these 53BP1 targeting strategies stimulate HDR without altering off-target editing by Cas9 or compromising cellular viability (Jayavaradhan et al., 2019; Nambiar et al., 2019; Paulsen et al., 2017). Additional studies have shown that combinatorial inactivation of 53BP1 along with upregulation of factors downstream of end resection enhances HDR rates. For example, co-expression of dn53BP1 and RAD52 additively enhances HDR using ssODN in human cells (Paulsen et al., 2017). This combination promotes end resection by blocking 53BP1 with dn53BP1 while stimulating SSTR by overexpressing RAD52 (Paulsen et al., 2017; Richardson et al., 2018; Shao et al., 2017). Likewise, fusions of the ubiquitin-binding domains of RAD18 or RNF169 to BRCA1, a key HDR factor required for RAD51 loading, have been shown to promote HDR, likely by blocking 53BP1, while promoting homologous recombination (Bashir et al., 2020). Finally, the possibility of activating HDR in the G1 phase has been demonstrated by restoring the BRCA1-PALB2-BRCA2 interaction (required for RAD51 loading) and activating CtIP-mediated end resection in G1 by expressing a phospho-mimetic CtIP in a 53BP1 knockout cell line (Orthwein et al., 2015). It remains to be investigated if this approach can promote HDR with sufficient efficiency and without cellular toxicity to enable editing of non-dividing cells for clinical applications. Moreover, because loss of 53BP1 is associated with hyper-resection and mutagenic repair by SSA (Ochs et al., 2016), the consequences of transiently inhibiting 53BP1 on genomic integrity need to be carefully evaluated.

In addition to 53BP1 regulators, proteins that stimulate end resection (e.g., CtIP and EXO1), DNA recombination factors (e.g., RAD52), and DNA polymerase subunits (e.g., POLD3) have been shown to promote HDR when fused to Cas9 (Table 1) (Charpentier et al., 2018; Hackley, 2021; Ma et al., 2020; Reint et al., 2021; Shao et al., 2017). Such Cas9 fusions can reduce the pleiotropic consequences of DDR manipulation caused by overexpression of DNA repair factors. Interestingly, phage-encoded single-strand DNA annealing proteins improve HDR 1,000-fold in bacteria through their interactions with host single-strand DNA binding proteins (Filsinger et al., 2021). However, it remains to be investigated whether the expression of recombination-promoting viral proteins could similarly stimulate HDR at Cas9-induced breaks in mammalian cells. Cell cycle modulation has also been shown to stimulate HDR by ensuring Cas9-mediated DSB formation at the HDR-permissive S and G2 phases of the cell cycle (Table 1) (Abe et al., 2020; Gu et al., 2018; Gutschner et al., 2016; Lin et al., 2014; Lomova et al., 2019; Wienert et al., 2020). Given that end joining pathways also repair DSBs in the S and G2 phases, inhibition of end joining has been shown to synergistically enhance HDR rates in combination with cell cycle modulation (Maurissen and Woltjen, 2020).

Emerging studies have also highlighted the role of the chromatin context in regulating DSB repair pathway choice. Indeed, Cas9 cleavage efficiency has been shown to correlate positively with chromatin accessibility and transcription of the target sequences (Chen et al., 2016, 2017b; Daer et al., 2017; Horlbeck et al., 2016; Jensen et al., 2017; Schep et al., 2021). It has also been observed that NHEJ-mediated repair of Cas9-induced DSBs is more efficient in euchromatin, while MMEJ and SSTR are more active in certain heterochromatin contexts (Schep

et al., 2021). Thus, besides modulating the activity of Cas9, the chromatin context can influence DSB repair pathway balance at Cas9-induced breaks (Chakrabarti et al., 2019; Schep et al., 2021). For example, inhibition of the H3K27 methyltransferase EZH2 enhances NHEJ-mediated DSB repair at H3K27me3-marked heterochromatin domains at the expense of MMEJ (Schep et al., 2021), while chromatinization of dsDNA donors has been reported to stimulate HDR events (Cruz-Becerra and Kadonaga, 2020). Future approaches that manipulate the chromatin context may provide new strategies to obtain specific DNA repair outcomes.

Undesirable DNA repair outcomes associated with Cas9-induced DSBs

DSBs are dangerous DNA lesions that can impair cell survival or lead to mutagenesis of the genome (Burgio and Teboul, 2020; Scully et al., 2019). The consequences of DSB formation include the generation of indels, gross chromosomal rearrangements (GCRs), and p53 activation. The nature of these undesirable outcomes associated with Cas9-induced DSBs are discussed below.

Mutational consequences of DSB induction and repair

Illegitimate and Undesirable DNA Donor Recombination. While HDR is primarily an error-free repair pathway, several lines of evidence suggest that exogenous donors can promote unintended mutagenesis at DSBs (Figure 2, bottom right). For example, dsDNA donors are prone to multiple head-to-tail integrations or concatenation at on- and off-target loci, which is often accompanied by indel formation, suggesting the involvement of NHEJ and MMEJ in these events (Paulis et al., 2015; Roberts et al., 2017; Skryabin et al., 2020; Zelensky et al., 2017). Additionally, inaccurate recombination events occurring using ssODNs have also been described (Boel et al., 2018; Gallagher et al., 2020; Paix et al., 2017; Rivera-Torres et al., 2017). These errors are attributed to mutagenic DNA synthesis during SSTR (Boel et al., 2018; Gallagher et al., 2020; Paix et al., 2017). Furthermore, it has also been shown that cellular cytidine deaminases can trigger the deamination of ssODN bases, resulting in base substitutions in genomic DNA (Lei et al., 2018).

Gross Chromosomal Rearrangements and Large Deletions. GCRs include chromosomal translocations, inversions, duplications, deletions, and other complex lesions associated with human cancers (Dahiya et al., 2021). Mounting evidence points toward the induction of complex chromosomal lesions by CRISPR-Cas nucleases (Figure 2, bottom) (Liu et al., 2021a). It was recently shown that DSBs introduced by Cas9 cause the formation of micronuclei and chromosome bridges, which subsequently result in catastrophic genomic rearrangements, such as chromothripsis (Leibowitz et al., 2021). The generation of micronuclei and chromosome bridges has been attributed to the presence of unrepaired Cas9-induced DSBs during cell division (Leibowitz et al., 2021). The formation of chromosomal rearrangements induced by chromothripsis can result from NHEJ, MMEJ, and HDR events (Hastings et al., 2009; Ly and Cleveland, 2017; Piazza et al., 2017; Rausch et al., 2012; Stephens et al., 2011). The generation of complex rearrangements is favored by the presence of multiple DSBs induced by site-specific nucleases at on- and off-target sites (Brunet and Jasin, 2018; Frock et al., 2015; Maddalo et al., 2014; Pristyazhnyuk et al., 2019).

Large genomic deletions (>250 bp) at single Cas9-induced DSBs have also been reported in mouse and human cell lines (Figure 2, bottom left) (Adikusuma et al., 2017, 2018; Alanis-Lobato et al., 2021; Cullot et al., 2019; Kosicki et al., 2018; Owens et al., 2019; Shin et al., 2017; Weisheit et al., 2020; Zuo et al., 2017). End resection and MMEJ factors have been implicated in the generation of large deletions (Kosicki et al., 2020; Owens et al., 2019). However, the size distributions of large deletions cannot be predicted by the location of microhomologies alone, thereby implicating multiple pathways in their generation. Genome editing in mouse and human embryos results in a high frequency of large deletions and complex rearrangements, causing loss of heterozygosity (Adikusuma et al., 2018; Alanis-Lobato et al., 2021; Egli et al., 2018; Papathanasiou et al., 2021; Zuccaro et al., 2020). The occurrence of these rearrangements has challenged recent observations of inter-homolog homologous recombination (IH-HR) following Cas9 cleavage in human embryos (Ma et al., 2017a, 2018), a process that is thought to be stimulated by RAD51 (Wilde et al., 2021). Indeed, targeting the *POU5F1* gene in human embryos caused a high frequency of large deletions that spanned 4 to 20 kb (Alanis-Lobato et al., 2021), while a single Cas9-induced DSB at a pericentromeric locus in human embryos was found to yield segmental and whole chromosome losses (Zuccaro et al., 2020). These studies highlight the risks of DSB-based genome editing in human embryos (Adikusuma et al., 2018; Alanis-Lobato et al., 2021; Egli et al., 2018; Zuccaro et al., 2020).

p53 activation and induction of apoptosis

DSBs activate the tumor suppressor p53, which preserves genome stability by triggering cell cycle arrest, cellular senescence, and apoptosis (Bieging et al., 2014). In genome editing applications, DSBs induced by Cas9 have been shown to cause p53-dependent cell cycle arrest in non-transformed human cells (Haapaniemi et al., 2018; Ihry et al., 2018). Surprisingly, mild, but sustained, p53 activation was also observed following expression of Cas9 itself without gRNA (Enache et al., 2020). These studies suggested that genome editing experiments may result in the selection of cell clones with a dysfunctional p53-dependent tumor suppressor response (Enache et al., 2020; Ylä-Herttuala, 2018), raising safety concerns for the use of edited cells in *ex vivo* therapeutic applications. Additional studies have shown that Cas9-induced p53 activation results in reduced proliferation and functional impairment of hematopoietic stem and progenitor cells, which could be partially overcome by transient p53 inhibition (Ferrari et al., 2020; Schiroli et al., 2019). In addition to p53 mutations, KRAS mutations have also been suggested to confer a selective advantage to cells undergoing Cas9-mediated DSB formation (Sinha et al., 2021). The toxicity induced by DSB formation is particularly apparent when high copy number loci or repetitive sequences are targeted (Aguirre et al., 2016; Castanon et al., 2020; Kuscu et al., 2017; Shen and Ideker, 2017). Interestingly, the cellular toxicity associated with targeting repeat sequences in the human genome has been exploited as a kill switch for Cas9-expressing cells (Castanon et al., 2020).

Future challenges of DSB-based genome editing

As previously discussed, DSB-based genome editing is influenced by the sequence context of the target locus. While the

main mutagenic outcome of Cas9-induced DSBs can be predicted (Allen et al., 2019; Chakrabarti et al., 2019; Chen et al., 2019; Leenay et al., 2019; Shen et al., 2018; Shou et al., 2018; van Overbeek et al., 2016), the precise mixture of indel outcomes of end joining pathways remains largely unpredictable. Recent high-throughput technologies to study the effects of genetic perturbations on DNA repair products (i.e., Repair-seq) have shown the high complexity of the repair events occurring at Cas9-induced DSBs and defined the end-processing enzymes responsible for the generation of specific DSB repair products (Hussmann et al., 2021). Future studies to define the influence of chromatin contexts and genetic backgrounds on the identity of the DSB repair products generated by DNA repair enzymes should provide a more comprehensive view of DSB repair events, opening potential avenues to modulate DSB repair for obtaining specific outcomes of interest.

DSB-based genome editing is also affected by the cellular and tissue context. However, there is currently limited understanding of the cell- and tissue-specific regulation of DSB repair events, limiting the implementation of CRISPR technologies for clinical applications (Ferreira da Silva et al., 2021a). In non-dividing cells (e.g., neurons), HDR is non-functional and installation of specific insertions into the genome has only been achieved through integration of DNA donors by end joining pathways (Suzuki and Izpisua Belmonte, 2018; Suzuki et al., 2016). However, because of the high frequency and indiscriminate nature of NHEJ, as well as the low fidelity of MMEJ, off-target integration of dsDNA donors used in such approaches remains a concern. Future work that elucidates the DSB repair processes occurring in terminally differentiated cells is needed to enhance the accuracy of end-joining-mediated precision editing.

In proliferating cells, HDR-mediated genome editing is the most precise pathway for DSB-based genome editing, as previously discussed. However, its relatively low frequency remains a major barrier for DSB-based precision genome engineering. DDR modulation is therefore a key strategy for stimulating DSB-based precision genome editing, particularly in contexts (e.g., *in vivo* editing) that do not allow for the enrichment of cells with desirable edits. As previously discussed, several DSB repair modulating strategies have been designed to stimulate HDR-based precision genome editing (Table 1). A comparative approach to evaluate the impact of these strategies on on-target or off-target editing is required to identify the most effective strategy in locus- or cell type-specific contexts. Additionally, future efforts are needed to develop approaches for the delivery of DNA repair modulators for precision genome editing applications *in vivo*.

SSB-BASED GENOME EDITING

SSB-based genome editing approaches rely on the use of CRISPR-Cas nickase mutant proteins. nCas9 proteins carry mutations in either the RuvC (e.g., D10A) or HNH (e.g., H840A, N863A) nuclease domains (Nishimasu et al., 2014). nCas9-based approaches have been utilized to generate single or paired nicks into the genome for end-joining-mediated gene disruption or HDR-dependent precision genome editing (Davis and Maizels, 2014; Mali et al., 2013; Ran et al., 2013), as discussed below.

Repair of CRISPR-induced single nicks

Single nicks can stimulate HDR-mediated precision genome editing, although with lower efficiency than DSBs (Davis and Maizels, 2014). Distinct from DSBs, nicks are asymmetric lesions that disrupt only one DNA strand. Multiple cellular repair mechanisms can stimulate nick-induced HDR using dsDNA donors or ssODNs (Figure 3, steps 1–7 and 10–13) (Maizels and Davis, 2018; Vriend and Krawczyk, 2017). Repair of nicks by HDR is more efficient on the coding strand relative to the non-coding strand (Davis and Maizels, 2014). Furthermore, nicks are repaired by ssODN-mediated HDR using strand-specific mechanisms (Davis and Maizels, 2014). ssODNs complementary to the nicked strand hybridize to the 3′ end of the nicked target, which then initiates DNA synthesis using the annealed donor as a template through SSTR-like mechanisms (Davis and Maizels, 2014, 2016; Maizels and Davis, 2018) (Figure 3, steps 2–9). On the other hand, ssODNs complementary to the intact DNA strand have been proposed to be directly incorporated into the genome (Davis and Maizels, 2014, 2016; Kan et al., 2017) (Figure 3, steps 10–15).

The repair of nCas9-induced nicks is controlled by DNA end resection factors and canonical HDR proteins in a manner dependent on the orientation of the nick and the use of dsDNA or ssODN donors (Maizels and Davis, 2018). While dsDNA donor-mediated HDR at nicks requires the RPA complex, BRCA2, and RAD51 (Davis and Maizels, 2014; Vriend and Krawczyk, 2017), ssODN-mediated genome editing at single nicks is inhibited by RAD51 and can occur independently from resection (Bothmer et al., 2017; Davis and Maizels, 2014; Maizels and Davis, 2018; Vriend and Krawczyk, 2017). Interestingly, the fusion of RAD51 mutants to nCas9 results in increased efficiency of ssODN-mediated HDR, while overexpression of wild-type RAD51 decreases ssODN-mediated HDR at nCas9-induced SSBs (Rees et al., 2019). ssODN-mediated HDR occurs with greater efficiency at nicks generated by Cas9 D10A than at Cas9 N863A-induced SSBs, possibly due to different accessibility of the DNA strand cleaved by the two nickase mutants (Bothmer et al., 2017). To further improve the efficiency and accuracy of Cas9 D10A-mediated genome editing, high-fidelity Cas9 D10A variants that display greater specificity and reduced off-targets have been derived (Wang et al., 2021b). Given the potential for accurate genome editing events promoted by Cas9 D10A, more fundamental studies dissecting the cellular mechanisms involved in the repair of nCas9-induced SSBs are needed.

Repair of paired nicks induced by Cas9 nickases

Paired nCas9 proteins targeting opposite DNA strands with PAM recognition sites facing outward with respect to each other (PAM-out orientation) have been shown to generate DSBs (Bothmer et al., 2017; Ran et al., 2013). Dual nicking by Cas9 D10A and N863A can generate DSBs with 5′ or 3′ overhangs, respectively (Bothmer et al., 2017). Cas9 D10A-induced DSBs with 5′ overhangs are repaired by HDR more efficiently relative to DSBs with 3′ overhangs induced by Cas9 N863A (Bothmer et al., 2017). Given that HDR requires the generation of 3′ ssDNA tails, DSBs with 5′ overhangs necessitate nucleolytic processing to initiate HDR. HDR events at Cas9 D10A-induced DSBs require RAD51 and BRCA2 when a dsDNA template is utilized for repair,

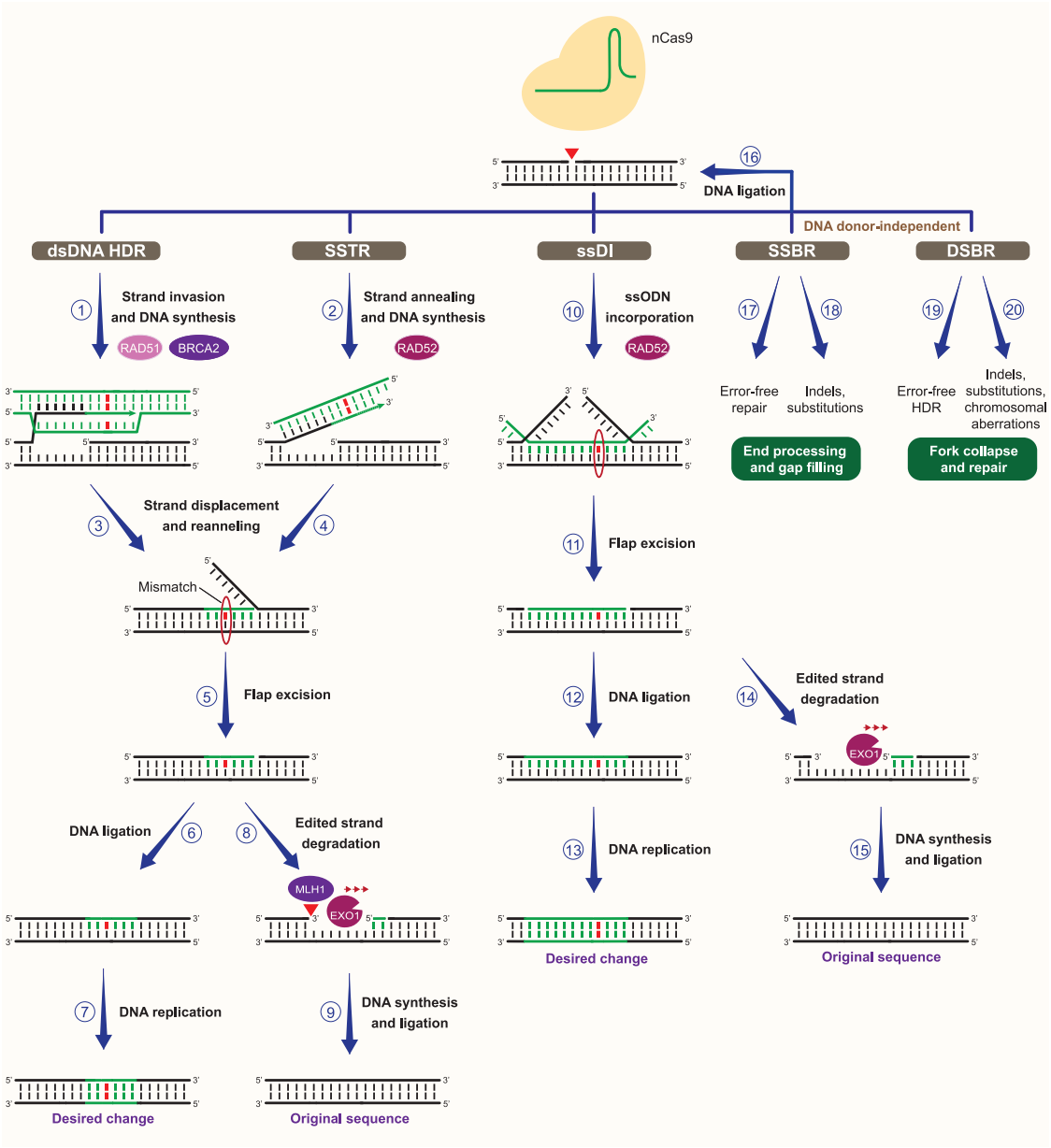

Figure 3. Repair of CRISPR-induced SSBs for precision genome editing

Multiple RAD51-dependent and -independent cellular mechanisms act at SSBs generated by nCas9 to promote the installment of genomic changes. In the presence of dsDNA donors, dsDNA donor-dependent HDR (dsDNA HDR) can lead to BRCA2- and RAD51-mediated invasion of the 3′ end of the nicked genomic DNA strand into the donor DNA, followed by templated DNA synthesis (1). ssODNs complementary to the nicked genomic DNA strand can instead serve as a template for DNA synthesis following RAD52-dependent ssODN annealing to the nicked strand through a SSTR-like process (2). Displacement of the genomic DNA strand from dsDNA or ssDNA donors and its reannealing to the parental strand can lead to the generation of a 5′ flap and the formation of heteroduplex DNA containing a mismatch between the edited and the parental sequence (3–4). Flap excision by 5′ flap endonucleases, followed by DNA ligation and DNA replication can then lead to the incorporation of the desired change (5–7). Annealing of ssODNs complementary to the non-nicked DNA strand through single-strand DNA incorporation (ssDI) can result in the formation of 5′ and 3′ flap structures and the generation of heteroduplex DNA with a mismatch (10). Incorporation of the desired change can then occur upon flap excision by 3′ and 5′ endonucleases, DNA ligation, and DNA replication (10–13). Recognition of mismatches between the edited and parental strand by the MMR machinery can cause EXO1-dependent degradation of the edited strand, restoring the original DNA sequence (8, 9, 14, and 15). Religation of the nCas9-induced nick can also result in the restoration of the original DNA sequence (16). Alternatively, DNA end processing and gap filling by SSB repair (SSBR) can lead to error-free repair or formation of indels and substitutions (17 and 18). Unrepaired SSBs can be converted into DSBs during DNA replication, resulting in the collapse of replication forks and their subsequent repair by either error-free HDR or error-prone DSB repair (DSBR), which can cause indels, substitutions, and chromosomal aberrations (19 and 20).

while they occur through SSTR-like, RAD51- and BRCA2-independent processes when using ssODNs (Bothmer et al., 2017). DSBs with 3′ overhangs generated by Cas9 N863A have, instead, been shown to undergo preferentially MMEJ-dependent repair, resulting in the generation of insertions (Bothmer et al., 2017). nCas9-induced DSBs can also be repaired through NHEJ (Ran et al., 2013). Distinct from paired nCas9 systems with PAM-out orientation, nCas9 strategies that employ gRNAs with PAM sites facing inward with respect to each other (PAM-in orientation) do not efficiently generate DSBs, possibly due to inadequate DNA strand separation, resulting in a low frequency of editing events (Bothmer et al., 2017; Mali et al., 2013; Ran et al., 2013).

Alternative strategies using paired nickases that generate nicks on the same DNA strand of the target site or on both the target site and a dsDNA donor molecule have been developed to promote HDR without the formation of DSBs (Chen et al., 2017a; Davis and Maizels, 2014; Gonçalves et al., 2012; Hyodo et al., 2020; Nakajima et al., 2018; Wang et al., 2021b). These nCas9-based strategies have been shown to display greater efficiency than single-nick-dependent approaches (Chen et al., 2017a; Davis and Maizels, 2014; Gonçalves et al., 2012; Hyodo et al., 2020; Nakajima et al., 2018), and in some instances they were reported to be as efficient as DSB-based strategies (Hyodo et al., 2020).

Applications and current limitations of SSB-based genome editing approaches

Paired nCas9 proteins that generate DSBs have been utilized for end-joining-mediated gene disruption or gene insertion in human cell lines and mouse zygotes and for HDR-dependent editing of human cell lines, including human embryonic stem cells (Bothmer et al., 2017; Mali et al., 2013; Ran et al., 2013). These approaches suffer from the same limitations discussed above for DSB-inducing nucleases. However, they display higher specificity and lower indel formation relative to wild-type Cas9-based approaches (Bothmer et al., 2017; Ran et al., 2013).

DSB-independent single- and dual-nick-based approaches have been utilized for generating gene mutations by HDR in human cell lines, including human embryonic stem cells and iPSCs (Bothmer et al., 2017; Chen et al., 2017a; Davis and Maizels, 2014; Gonçalves et al., 2012; Hyodo et al., 2020; Li and Margolis, 2021; Nakajima et al., 2018; Rees et al., 2019; Wang et al., 2021b). nCas9-based approaches have also been employed in human cell lines to induce the contraction of trinucleotide repeats associated to neurological diseases (Cinesi et al., 2016). DSB-independent nick-based strategies do not lead to the activation of p53-dependent responses associated with DSB-inducing nucleases (Hyodo et al., 2020). While nicks are significantly less toxic lesions than DSBs, the introduction of hundreds of nicks into the genome can reduce cellular viability (Smith et al., 2020). Thus, the impact of off-target nicking by nCas9 needs to be further explored, and such studies could benefit from recently developed assays that efficiently capture SSBs in genomes (Cao et al., 2019, 2020; Elacqua et al., 2021; Sriramachandran et al., 2020). In addition, processing of nCas9-induced SSBs can lead to indels and nucleotide substitutions (Figure 3, step 18), which are suppressed by RAD51 and BRCA2 and depend on DNA2-mediated gap extension and Polθ-dependent mutagenic DNA synthesis (Bothmer et al., 2017; Davis and Maizels, 2014; Zhang et al., 2021). In some instances, nicks introduced by genome editing machineries can also be converted into DSBs, causing mutagenic events. For example, when replication forks encounter a nick, they can convert it into a one-ended DSB (Kuzminov, 2001), resulting in replication fork collapse (Figure 3, step 20). Alternatively, endogenous cytidine deaminases can cause DSB formation by generating additional nicks on the opposite strand of the nCas9-induced SSB (Lei et al., 2018). However, single nicks introduced by Cas9 nickases lead to limited formation of indels relative to DSBs generated by wild-type Cas9 or paired nCas9 (Bothmer et al., 2017; Davis and Maizels, 2014; Rees et al., 2019; Zhang et al., 2021), suggesting a reduced contribution of error-prone mechanisms to the repair of nCas9-induced single nicks. Given their overall higher fidelity, SSB-based HDR strategies may prove to be more attractive than DSB-based HDR approaches for precision genome editing applications that require greater accuracy (e.g., clinical applications).

DNA DEAMINATION-MEDIATED GENOME EDITING

Direct modification of nucleotides in DNA sequences or RNA transcripts can be achieved through base editing (Rees and Liu, 2018). Here, we focus on DNA base editing, which utilizes two main types of editing machineries: adenine base editors (ABE) and cytosine base editors (CBE) (Molla and Yang, 2019; Porto et al., 2020). We refer readers interested in RNA base editing to recent reviews covering this topic (Pickar-Oliver and Gersbach, 2019; Rees and Liu, 2018).

Development and architecture of base editors

Base editors are programmable molecular machines that deaminate base(s) at targeted loci within a window of high activity (Anzalone et al., 2020; Huang et al., 2021b) without introducing DSBs and with limited activation of DDR signaling or induction of apoptosis (Kuscu et al., 2017; Wang et al., 2020c). To achieve potent site-specific and targeted base deamination, base editors harbor multiple modules with distinct functions required for (1) localization to desired genomic regions, (2) chemical modification of targeted DNA base(s), and (3) inhibition of key DNA repair processes (only for CBE). Base editors use the programmable genome search and targeting ability of CRISPR systems to locate and engage the desired targeted base(s). Similar to other CRISPR systems, genomic targeting of base editors requires the formation of a three-stranded RNA-DNA structure (R-loop) between the gRNA and the genomic sequence complementary to the gRNA (Figure 4) (Lapinaite et al., 2020). Base editors also contain natural or engineered ssDNA-specific deaminases that modify bases within ssDNA exposed by the R-loop structure, thereby exhibiting activity on a restricted window of accessible genomic bases. In particular, CBE consists of a cytidine deaminase (e.g., APOBEC1/3, AID, or CDA) fused to a dCas9 or a nCas9 with an active HNH nuclease domain (Figure 4) (Hess et al., 2016; Komor et al., 2016; Ma et al., 2016; Nishida et al., 2016). Cytidine deaminases play critical roles in promoting mutagenesis during innate and adaptive immunity (Conticello et al.,

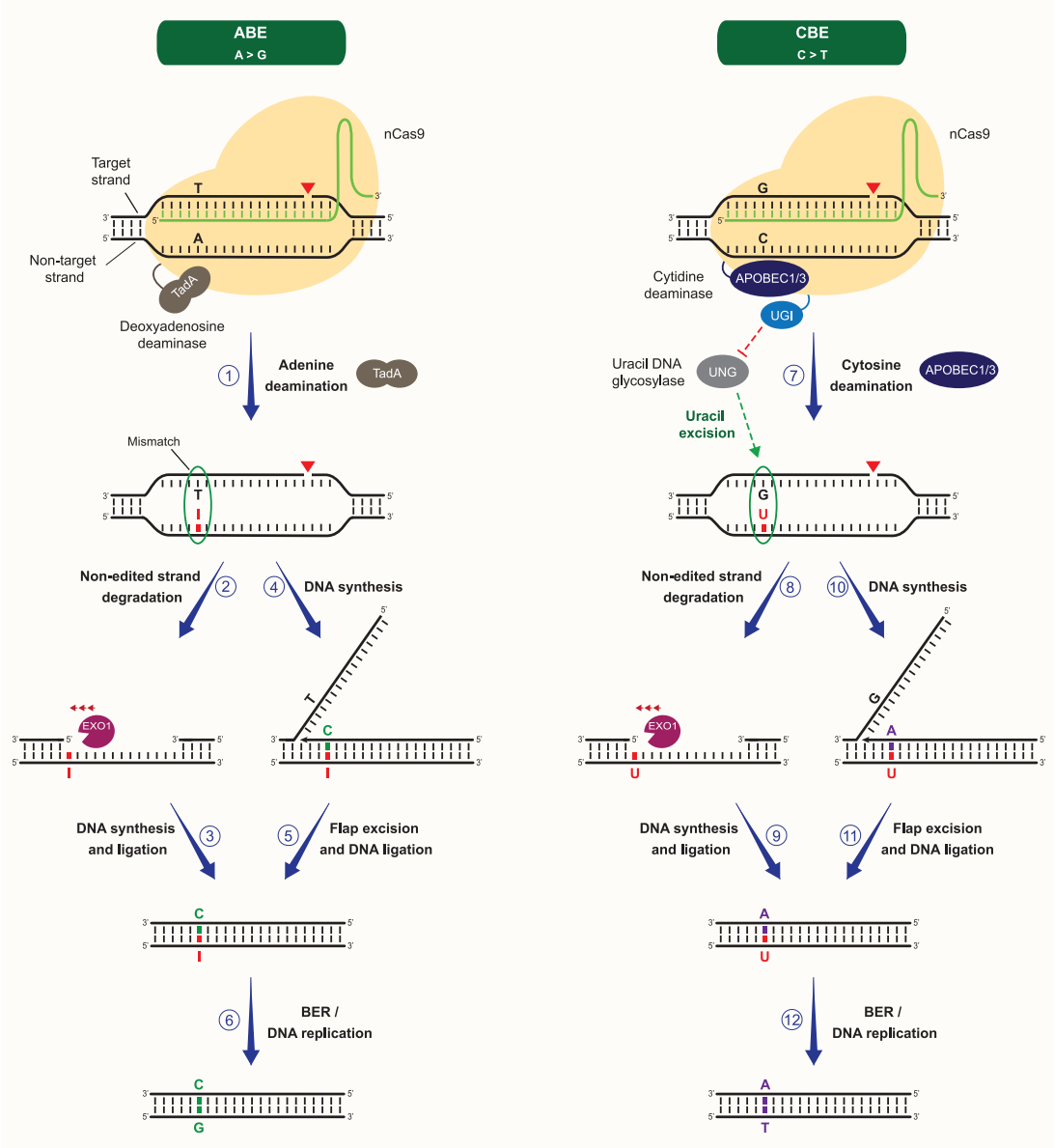

Figure 4. Repair of site-specific base lesions generated by canonical base editors

Generation of site-specific base transitions by the canonical base editors ABE and CBE. Traditional ABE is constituted of a fusion of nCas9 to the TadA deoxyadenosine deaminase. ABE promotes the deamination of adenines located within the ssDNA of the R-loop generated upon gRNA pairing to the targeted DNA strand (1). Adenine deamination leads to the formation of inosine and the generation of a I:T mismatch, which can be recognized by the MMR machinery (1). nCas9-dependent nicking of the non-edited strand can promote its degradation by EXO1 and its subsequent resynthesis using the edited strand as a template, resulting in the incorporation of a cytosine opposite to inosine (2 and 3). Alternatively, replacement of the T with a C can occur upon DNA synthesis initiated by the 3′ end of the nicked non-edited strand, followed by strand displacement, excision of the resulting 5′ flap and DNA ligation (4 and 5). These events can occur in a MMR-dependent manner or result from long-patch repair of the nCas9-induced SSB. T to C substitution can also occur following religation of the nicked strand and DNA replication (not shown). Subsequent DNA replication or BER can lead to the replacement of the I with a G and the generation of a A:T->G:C transition (6). Distinct from ABE, CBE is traditionally constituted of a fusion of nCas9 to the cytidine deaminase APOBEC1/3, which catalyzes the deamination of cytosines into uracils within the ssDNA of the gRNA-containing R-loop (7). CBE inhibits uracil excision by UNG through a UGI peptide fused to nCas9, resulting in the persistence of a G:U mismatch, which can be recognized by MMR proteins (7). Similar to ABE, CBE then catalyzes the nicking of the non-edited strand, favoring its degradation or its displacement and excision, followed by the replacement of the G with an A upon DNA synthesis (7–11). G to A substitution can also occur upon DNA replication (not shown). Subsequent DNA replication or BER can lead to the replacement of the U with a T and the generation of a C:G->T:A transition (12).

2007) and are also responsible for a significant fraction of the genomic mutations observed in human cancers (Buisson et al., 2019; Burns et al., 2013; Nik-Zainal et al., 2012; Roberts and Gordenin, 2014). CBEs divert the mutagenic activity of ssDNA-specific cytidine deaminases toward genomic bases of interest within the R-loop. Because natural enzymes that deaminate adenosine in ssDNA are not currently known, ABEs were derived as a result of protein engineering and directed evolution

experiments. In particular, Gaudelli et al. (2017) evolved the prokaryotic tRNA-specific adenosine deaminase TadA (Wolf et al., 2002) to acquire a deoxyadenosine deaminase activity that can operate on ssDNA (Figure 4). Of note, CBEs, but not ABEs, harbor an additional module that functions as a uracil DNA glycosylase inhibitor (UGI). UGI is a DNA mimic peptide isolated from PBS2, a unique bacteriophage that has a genome rich in uracil instead of thymine residues (Takahashi and Marmur, 1963). UGI potently inhibits UNG, the major DNA glycosylase involved in the removal of uracil from DNA (Wang and Mosbaugh, 1989). During base editing, UNG inhibition allows uracil intermediates generated by CBE to persist in genomic DNA, enabling the subsequent engagement of DNA repair pathways that lead to base substitution, as discussed below.

Mechanisms of base editing-dependent lesion repair

Base deamination can result from exposure to environmental DNA damaging agents, occur spontaneously upon DNA decay, or be induced by the action of cellular deaminases and base editors. Hydrolytic deamination of the exocyclic amine of adenine or cytosine yields the non-canonical inosine or uracil bases, respectively (Alseth et al., 2014; Nabel et al., 2012). During DNA replication, inosine is read by DNA polymerases as guanine, generating I:T to I:C substitutions, while uracil is read as thymine, causing U:G to U:A changes.

Modified bases are normally detected, excised, and replaced by the BER pathway (Beard et al., 2019; Caldecott, 2020). BER is initiated by specialized DNA glycosylases, which catalyze the cleavage of the glycosidic bond between the nitrogenous base and the deoxyribose sugar of specific damaged nucleotides, thereby generating an abasic site (also known as an apurinic/apyrimidinic site or AP site) (Krokan and Bjørås, 2013). Abasic sites are among the most prevalent DNA lesions in the genome, and it is estimated that ~10,000 abasic sites are formed in every cell of the human body each day (Barnes and Lindahl, 2004). If left unrepaired, abasic sites threaten genomic integrity, as they can interfere with DNA replication and transcription (Thompson and Cortez, 2020). In mammalian cells, uracil DNA glycosylases, such as UNG and SMUG1, prevent genomic instability by removing uracils. UNG, the main uracil DNA glycosylase, has two isoforms, UNG1 and UNG2, that remove uracil from mitochondrial and nuclear DNA, respectively (Krokan and Bjørås, 2013). Distinct from uracil, inosine is thought to be excised from DNA by the AAG glycosylase (Alseth et al., 2014). Abasic sites generated by DNA glycosylases are subsequently processed by AP site DNA lyases (AP lyases), such as APEX1 or APEX2, with help from other BER factors, such as PARP1 and XRCC1 (Beard et al., 2019). XRCC1 acts as scaffold for BER factors and regulates PARP1, preventing BER misregulation and genomic instability (Demin et al., 2021; Lindahl, 2000). APEX1 is the main AP lyase that initiates abasic site repair by incising the phosphate backbone on the 5′ side of the abasic site to create a SSB (Beard et al., 2019). *De novo* DNA synthesis by DNA polymerases then fills the remaining gap using the undamaged strand as a template. Two main SSB repair pathways that differ in the length of DNA synthesis promote the filling of gaps resulting from the processing of DNA base or backbone lesions: short-patch repair promotes the synthesis of a single nucleotide by the DNA polymerase β, while long-patch repair leads to the synthesis of 2–12 nucleotides catalyzed by the DNA polymerases β, δ, or ε (Caldecott, 2014). During long-patch repair, a 5′ ssDNA flap structure is generated by strand displacement DNA synthesis and then subsequently resolved by the FEN1 endonuclease (Prasad et al., 2001). Finally, DNA ligase I or III seals the remaining nick in the phosphate backbone, restoring the integrity of the double helix (Cappelli et al., 1997; Prasad et al., 1996).

Excision of inosine and uracil by the BER pathway reestablishes the original sequence and, therefore, counteracts the activity of base editors. Consequently, efficient base editing requires inhibiting BER to stabilize the modified base(s). As discussed above, in the case of cytosine deamination by CBE, this effect is achieved by inhibiting UNG using the UGI peptide (Figure 4). Interestingly, the fusion of one or multiple UGI peptides to CBEs and the ectopic expression of UGI enhance base conversion efficiency by blocking BER initiation (Komor et al., 2016, 2017; Wang et al., 2017). In ABE experiments, strategies to inhibit inosine excision are not required, given the limited rate of inosine removal by cellular glycosylases. Indeed, it was shown that cells lacking AAG or expressing fusions of ABE to inactive forms of human AAG or *E. coli* EndoV to block cellular AAG from accessing inosine do not improve ABE-mediated editing efficiency (Gaudelli et al., 2017). These results suggest that AAG is inefficient at excising inosine from genomic DNA, possibly because inosine is not frequently found in genomic DNA, given the absence of cellular deoxyadenosine deaminases. Consequently, ABE-mediated deoxyadenosine deamination leads to limited engagement of BER, thus yielding base editing products of high purity.

The U:G or I:T mismatches generated by base editors can be recognized and resolved by MMR (Figure 4, steps 1–3 and 7–9), a pathway that corrects errors introduced by DNA polymerases during DNA replication (Sanders et al., 2021). MMR removes mismatches by catalyzing the degradation of the newly synthesized DNA strand containing the incorrect base and restoring the correct sequence using the parental DNA strand as a template (Jiricny, 2013). To repair mismatches, MMR must distinguish the correct from the incorrect DNA strand. In eukaryotes, strand discrimination is achieved through the recognition of SSBs and gaps occurring on the newly synthesized DNA strand during DNA replication (Cortez, 2019; Hsieh and Zhang, 2017; Kunkel and Erie, 2015; Schanz et al., 2009). SSBs and gaps on the 5′ side of the mismatch provide entry points for EXO1-mediated DNA degradation, resulting in the processing of the newly synthesized DNA strand and the removal of the incorrect DNA base (Constantin et al., 2005; Goellner et al., 2015; Zhang et al., 2005). Gap filling by the replicative DNA polymerases δ or ε, followed by ligation by DNA ligase I, then restores DNA integrity. To promote the removal of the non-edited base of the mismatch by MMR, CBE and ABE fusions containing nCas9 introduce a nick on the 5′ side of the mismatch on the non-edited strand, thus favoring its degradation by EXO1 (Figure 4, steps 2 and 8) (Gaudelli et al., 2017; Gu et al., 2021; Komor et al., 2016). DNA synthesis can then result in the incorporation of an A opposite to U or a C opposite to I (Figure 4, steps 3 and 9). This process can also occur through a round of DNA replication or following DNA synthesis initiated by the 3′ end of the nick,

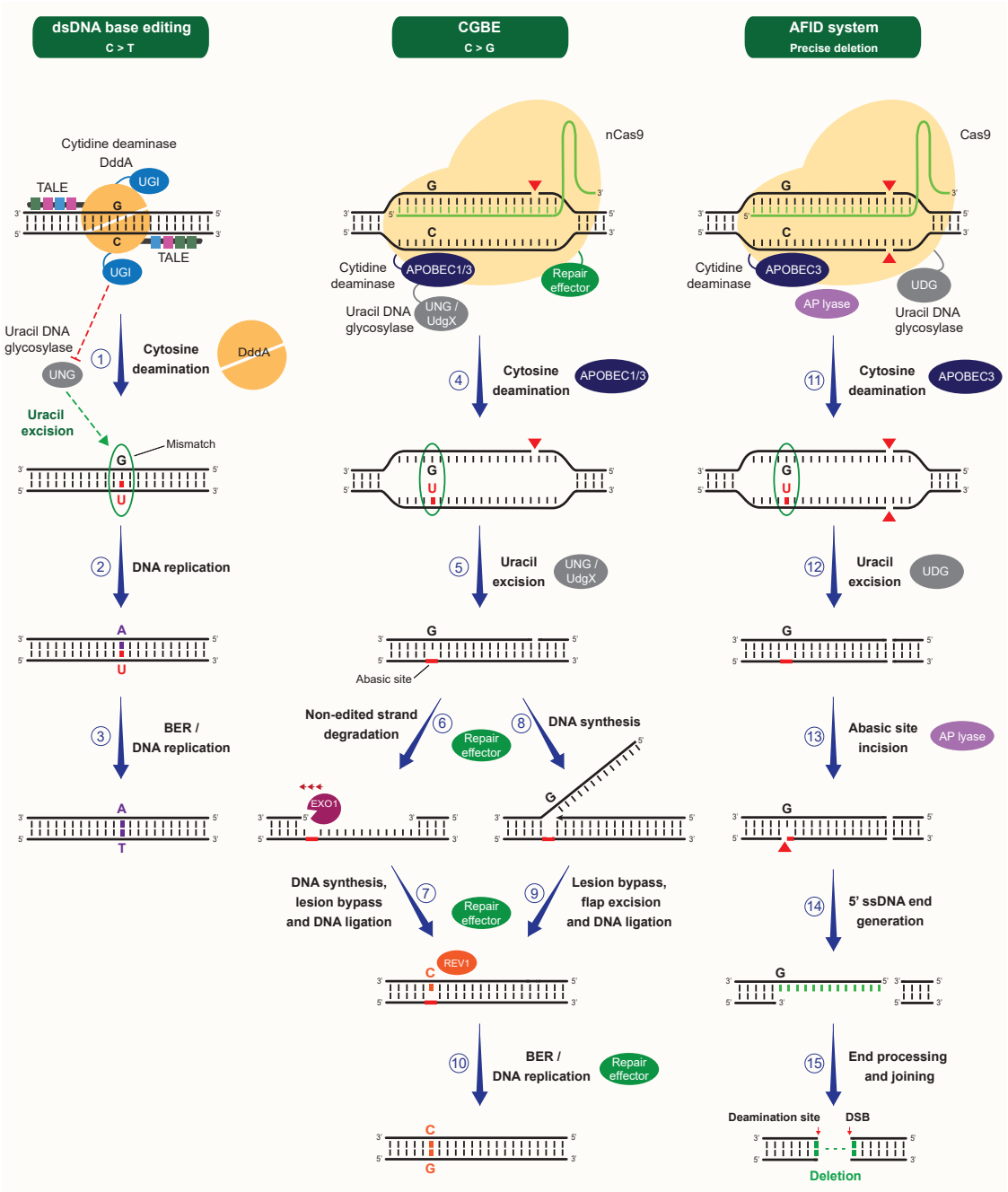

Figure 5. Repair of site-specific base lesions generated by non-canonical base editors

Modification of dsDNA and generation of transversion mutations or predictable deletions by non-canonical base editors (DddA, CGBE, and AFID systems). dsDNA-dependent base editing in the mitochondrial genome can be obtained using a split DddA deaminase fused to TALE arrays, which provide site-specific DNA binding. Reconstituted DddA deaminates cytosines located at the TALE DNA binding site, generating uracil and inhibiting its excision through a UGI peptide fused to DddA (1). The resulting G:U mismatch can then be resolved through DNA replication and BER events, leading to the generation of a C:G->T:A transition (2 and 3). CGBE enzymes are derived from CBE, whereby the UGI peptide has been removed or replaced with the uracil DNA glycosylase UNG or UdgX. Similar to CBE, CGBE promotes site-specific cytosine deamination and nicks the non-edited strand (4). However, in the case of CGBE, the uracil is then excised by uracil DNA glycosylases, resulting in the formation of an abasic site (5). The non-edited strand can then be degraded by EXO1 and undergo resynthesis by the replicative DNA polymerases δ or ε (6 and 7). Upon encountering the abasic site, the replicative DNA polymerases are replaced by translesion DNA polymerases, such as REV1, which can insert a cytosine opposite to the abasic site (7). Insertion of a C opposite to the abasic site can also occur upon DNA synthesis initiated by the 3′ end of the nicked non-edited strand, followed by the bypass of the abasic site by translesion DNA polymerases and the excision of the 5′ flap resulting from strand displacement DNA synthesis (8 and 9). DNA replication or abasic site repair by AP lyases and other BER enzymes can then lead to the replacement of the abasic site with a G, resulting in a C:G->G:C transversion (10). The generation of transversion mutations is stimulated by DNA repair effectors fused to CGBE (6–10). AFID systems are based on a fusion of Cas9 to APOBEC3 and a bacterial uracil DNA glycosylase. Similar to CGBE, AFID systems promote cytosine deamination into

(legend continued on next page)

accompanied by the displacement of the non-edited strand and the excision of the resulting 5′ flap (Gu et al., 2021) (Figure 4, steps 4, 5, 10, and 11). Nick-induced DNA synthesis can take place upon recognition of the mismatch by the MMR machinery or result from long-patch repair of the nCas9-induced SSB. Subsequent DNA replication or BER would then replace the U and I with T and G, respectively, leading to C:G->T:A and A:T->G:C base transitions (Figure 4, steps 6 and 12) (Gaudelli et al., 2017; Komor et al., 2016; Nishida et al., 2016).

Although base editing can also yield byproducts, such as indels and transversion mutations, these outcomes are generated with relatively low frequency at the targeted locus. Indels originate from end-joining-mediated repair of DSBs (Koblan et al., 2021a), likely resulting from nCas9-mediated nicking of the non-edited strand and APEX1-dependent nicking of the edited strand at abasic sites generated upon removal of the edited bases by DNA glycosylases. Additionally, DNA synthesis across abasic sites by error-prone translesion synthesis (TLS) polymerases can lead to the insertion of incorrect bases, thereby generating transversion mutations (Koblan et al., 2021a). Indeed, by modulating the formation of BER intermediates during base editing, non-canonical base editors that generate predictable transversion mutations and small deletions have recently been developed.

Generation of transversion mutations and precise deletions using non-canonical base editors

As discussed above, canonical base editors generate transition mutations, with transversion mutations occurring occasionally as byproducts. Although the generation of transversion mutations by base editors can be predicted from machine learning models (Arbab et al., 2020; Marquart et al., 2021), it remains infrequent and context-specific. Importantly, genetic deletion of *UNG* or addition of multiple UGI peptides to base editors leads to a reduced formation of transversion mutations, suggesting that these events might result from the accumulation of abasic sites due to enhanced removal of uracil (Komor et al., 2016, 2017; Kurt et al., 2021).

In mammalian cells, abasic sites can be shielded from DNA processing enzymes through crosslinking to HMCES (Mohni et al., 2019). Alternatively, abasic sites can be bypassed using HDR or TLS (Thompson and Cortez, 2020). In particular, the TLS polymerase REV1, which exhibits an intrinsic dCMP transferase activity, incorporates cytosines across abasic sites, thereby leading to C:G->G:C transversion mutations following cytosine deamination and uracil removal (Chan et al., 2013; Kim et al., 2011; Lin et al., 1999; Nelson et al., 1996). Accordingly, loss of REV1 was reported to decrease the formation of C:G->G:C transversions during AID-mediated somatic hypermutation in mice (Jansen et al., 2006). Therefore, stimulation of REV1-mediated DNA synthesis across abasic sites could potentially increase the frequency of C:G->G:C transversions. These findings suggest that stabilization of abasic sites generated from uracil excision may favor error-prone DNA repair events and enhance the generation of transversion mutations by base editors. Based on these observations, novel base editors that exploit the repair of abasic sites have been developed to generate C:G->G:C transversions (Figure 5, steps 4–10). These non-canonical base editors, named CGBEs for "C-to-G base editors," consist of a modified CBE in which the UGI has been removed or replaced by a uracil DNA glycosylase, such as UNG (Kurt et al., 2021; Zhao et al., 2021b) or UdgX (Koblan et al., 2021a). In certain CGBEs, nCas9 has also been fused to DNA repair effectors, such as the BER factors XRCC1, DNA ligase III and DNA polymerase β, RBMX, EXO1, and POLD2 (Chen et al., 2021a; Koblan et al., 2021a). The observation that ABE catalyzes the deamination of cytosine in a restricted sequence context (TC motifs) in human cells (Kim et al., 2019) has also led to the derivation of an ABE variant with increased C:G to G:C editing at TC sequences (Jeong et al., 2021). Therefore, a set of CGBEs displaying complementary features based on sequence context preference, editing purity, and efficiency are currently available for genome editing studies.

Recently developed non-canonical base editing approaches also include the APOBEC-Cas9 fusion-induced deletion (AFID) system, which introduces and processes uracil bases to generate small predictable targeted deletions (Wang et al., 2020b). The AFID system combines a Cas9 nuclease, a cytidine deaminase, a uracil DNA glycosylase, and an AP lyase in a single construct (Figure 5, steps 11–15). The target base(s) is deaminated into uracil(s) by the cytidine deaminase. The uracil DNA glycosylase then generates an abasic site, which is subsequently nicked by the AP lyase. Additionally, Cas9 cuts the two strands on the 3′ side of the nick, thus exposing the ssDNA region in between the nick and the DSB. These steps generate two non-compatible DNA ends (blunt end and ssDNA-containing end), requiring nucleolytic degradation of the ssDNA region before subsequent blunt ligation of the two ends by NHEJ. Therefore, AFID systems generate small deletions spanning from the deaminated C to the Cas9 cleavage site (Wang et al., 2020b). Improvements in the prediction of cytosine deaminase activity based on the sequence context, combined with the use of Cas9 PAM variants appropriate for the locus of interest, should enable a more precise generation of desired small deletions, which would be particularly useful for generating in-frame protein deletion mutants. Additionally, the accuracy of the deletions generated by AFID could be further improved by modulating the activity of the DNA repair factors fused to Cas9. Future work will be required to optimize the architecture of non-canonical base editors to maximize the generation of C:G->G:C transversions and small deletions and enhance product purity in different cellular systems.

Base editing on dsDNA

Canonical base editors are unable to edit bases in the context of dsDNA because of the ssDNA-specific activity of cytidine and

uracil, which is then excised by the uracil DNA glycosylase, generating an abasic site (11 and 12). However, distinct from CGBE, AFID systems introduce a DSB near the PAM sequence and express an AP lyase, which introduces a nick at the abasic site, resulting in the generation of a DNA end with a 5′ ssDNA tail (13 and 14). Degradation of the 5′ ssDNA tail and DSB repair by NHEJ results in predictable deletions spanning from the deaminated cytosine to the site of the Cas9-induced DSB (15).

adenosine deaminases. Furthermore, because RNA molecules cannot enter mitochondria, the mitochondrial genome is not a substrate for conventional CBE and ABE editors (Gammage et al., 2018). However, recent studies have shown that DddA, a toxin secreted by bacteria to induce replication stress and genomic instability in neighboring bacteria, displays cytidine deaminase activity on dsDNA (de Moraes et al., 2021). To determine whether DddA could be used for base editing on dsDNA, its toxic deaminase domain was split into two non-toxic halves and fused to two TALE arrays, which enables the *in situ* reconstitution of a functional cytidine deaminase domain upon DNA binding (Mok et al., 2020). The resulting DddA-derived cytosine base editor (DdCBE) was shown to efficiently convert C:G to T:A in nuclear dsDNA of mammalian cells, and similar editing events were observed in mitochondrial dsDNA following the fusion of DdCBE to a mitochondrial-targeting signal (Mok et al., 2020) (Figure 5, steps 1–3). Although lacking DSB repair, mitochondria possess functional BER and MMR pathways that enable the repair of base lesions generated by base editing (Fu et al., 2020). Accordingly, fusion of one or two UGI peptides to DdCBE was shown to enhance its editing efficiency and decrease base editing byproducts, probably through inhibition of UNG1. Besides its use for editing the mitochondrial genome in mammalian cells, DdCBE has also been successfully exploited to modify mitochondria in mice and zebrafish embryos (Lee et al., 2021; Sabharwal et al., 2021). The use of DdCBE has enabled the direct manipulation of mitochondrial DNA (mtDNA) in living systems with single-base resolution without requiring the formation of R-loop structures necessary for the activity of ssDNA-specific deaminases and in the absence of mitochondrial DSBs, which cause mtDNA degradation and potent induction of innate immune signaling events (Tigano et al., 2021). The development of DdCBE is a prime example of how mining bacterial genomes can lead to the discovery of unexpected enzymatic activities that can be harnessed to create new genome editing tools. Further advancements in the generation of base editing tools for mtDNA will provide novel opportunities to correct pathogenic mtDNA variants that cause mitochondrial disorders (Murphy and Hartley, 2018) and will enable a better understanding of mtDNA repair mechanisms.

Applications of canonical and non-canonical base editors

Base editors have been successfully applied to introduce targeted genomic modifications in many cellular and animal models (Molla and Yang, 2019). Furthermore, base editing has allowed investigators to model and correct genetic variants in both dividing and non-dividing cells (Billon et al., 2020; Gaudelli et al., 2017; Komor et al., 2016; Newby and Liu, 2021; Yeh et al., 2018), inactivate genes through the insertion of premature STOP codons (Billon et al., 2017; Kuscu et al., 2017) or splice site mutations (Gapinske et al., 2018; Kluesner et al., 2021; Yuan et al., 2018), disrupt multiple loci without the formation of undesired chromosomal translocations and mosaicism (Kim et al., 2017a; Webber et al., 2019), and study coding and non-coding nucleotide variants at scale (Cheng et al., 2021; Cuella-Martin et al., 2021; Després et al., 2020; Hanna et al., 2021; Hess et al., 2016; Huang et al., 2021a; Jun et al., 2020; Kweon et al., 2020; Li et al., 2018a; Ma et al., 2016; Sangree et al., 2021; Xu et al., 2021a). With all three modes of currently available base editors, CBE (C:G->T:A), ABE (A:T->G:C), and CGBE (C:G->G:C), a complete set of mutation types can be theoretically obtained by multistep editing (Zhao et al., 2021b). For example, the target C of C:G can be initially edited into U by CBE (U:G), and U:G can in turn be converted into T:A by cellular DNA replication and repair mechanisms. Alternatively, C:G can be edited into G:C by CGBE, followed by CBE-mediated conversion of the nascent G:C pair into A:T. This strategy could then convert a targeted C into T (CBE), G (CGBE), or A (CGBE + CBE). Similarly, A can be edited into G (ABE), C (ABE + CGBE), or T (ABE + CGBE + CBE). However, multi-step editing is difficult to achieve at high efficiency and specificity because of the limitations of base editing discussed below. Given its ability to precisely induce base transition mutations at single-nucleotide resolution, base editing has been employed to generate new cellular and animal models of cancer and various genetic syndromes (Annunziato et al., 2020; Rosello et al., 2021; Ryu et al., 2018; Zafra et al., 2018). Especially promising is the prospect of using base editing to revert disease-causing mutations (Doudna, 2020; Porto et al., 2020). For example, base editing has already been applied to rescue animal models of Hutchinson-Gilford progeria syndrome, sickle cell disease, tyrosinemia, DMD, phenylketonuria, and deafness, andto reduce cholesterol levels in mice and primates (Koblan et al., 2021b; Levy et al., 2020; Musunuru et al., 2021; Newby et al., 2021; Rossidis et al., 2018; Rothgangl et al., 2021; Ryu et al., 2018; Song et al., 2020; Villiger et al., 2018; Yeh et al., 2020). These remarkable pre-clinical results bode well for future applications of base editing in the treatment of many human diseases.

Current limitations of base editing approaches

Despite its high efficiency, base editing is restricted to a window of high deaminase activity (~5–8 bp for Cas9-based CBE and ABE) located at a specific position from the PAM sequence. Furthermore, the editing outcome and efficiency of base editing can be affected by the sequence context of the targeted locus. The presence of multiple editable bases within the activity window—and occasionally outside of the window—can result in undesired bystander mutations. Although prediction methods for the outcome and efficiency of base editing guides have been developed based on studies in HEK293 and mouse ES cells (Arbab et al., 2020; Marquart et al., 2021; Wang et al., 2021c), these methods require further refinement through studies in other cellular systems.

To overcome the above limitations, base editors have been extensively engineered for improving their targeting scope, efficiency, and specificity (Anzalone et al., 2020; Arbab et al., 2020; Fu et al., 2021; Gaudelli et al., 2020; Gehrke et al., 2018; Grünewald et al., 2020; Hu et al., 2018a; Jeong et al., 2021; Kim et al., 2017b; Kleinstiver et al., 2019; Koblan et al., 2018; Li et al., 2018b; Richter et al., 2020; Sakata et al., 2020; Walton et al., 2020; Wang et al., 2018, 2021a; Zafra et al., 2018; Zhang et al., 2020a, 2020b). These studies have led to the development of a large set of base editors with distinct PAM requirements and editing windows, higher on-target editing efficiency, and decreased generation of off-target events. In addition, to generate concomitant A:T->G:C and C:G->T:A changes, base editors with both ABE and CBE activities have been developed

(Grünewald et al., 2020; Li et al., 2020a; Sakata et al., 2020; Zhang et al., 2020b). However, there are currently no tools available to simultaneously introduce combinatorial transition and transversion mutations. It is expected that future developments will provide a greater array of base editing tools with multiple distinct features, allowing for even more precise genomic changes, including combinations of selected mutations, in the locus and cell type of interest. At the same time, given that the SSBs and abasic sites generated during base editing can potentially interfere with DNA replication and transcription, additional studies of the impact of base editors on genomic stability in the cell type of interest would be especially important.

ERROR-PRONE DNA-SYNTHESIS-BASED GENOME EDITING

DNA synthesis promoted at loci of interest enables the rewriting of genomic DNA sequences for precision genome editing. As discussed above, HDR-mediated genome editing strategies entail the copying of a synthetic donor template by cellular DNA polymerases at sites of DSB or SSB formation. Additionally, certain non-canonical base editors exploit error-prone TLS polymerases to insert cytosines across abasic sites and generate transversion mutations. Recent studies have successfully employed a nCas9 fusion to PolI3M, an *E. coli* DNA polymerase PolI mutant engineered for low fidelity, to initiate DNA synthesis from nCas9-induced nicks in the genomes of both bacteria and yeast (Halperin et al., 2018; Tou et al., 2020). Although this system, known as EvolvR, is efficient in generating a spectrum of transition and transversion mutations, the identity of the mutations introduced is not predictable. Furthermore, it remains to be established whether EvolvR, or a similar approach that relies on nCas9-fusions to other error-prone DNA polymerases, can be successfully applied to mammalian systems.

REVERSE-TRANSCRIPTION-BASED GENOME EDITING

Mechanism of prime editing

Reverse transcriptases (RTs) synthesize complementary DNA strands from an RNA template, transferring genetic information from RNA to DNA molecules (Martín-Alonso et al., 2021). Recent studies have exploited the activity of a CRISPR-based engineered RT paired with a multifunctional prime editing gRNA (pegRNA) to introduce sequences of interest into the genome without DSB intermediates or exogenous DNA templates (Anzalone et al., 2019). This technology, known as prime editing, leverages the possibility to extend the 3′ end of gRNAs with sequences of interest and exploits the ability of nicked DNA strands to prime DNA synthesis by RTs when annealed to RNA templates of interest. Prime editors are composed of a modified Moloney murine leukemia virus (M-MLV) RT conjugated to nCas9 (Figure 6A). The pegRNA carries a reverse transcription template that encodes the desired sequence to be incorporated in the genome, a primer binding site (PBS) to prime the RT, and a regular gRNA sequence for nCas9-mediated genomic targeting. The nick introduced by nCas9 (HNH mutant) on the non-target strand enables pairing of the generated 3′ ssDNA end with its complementary sequence located within the PBS. This DNA:RNA heteroduplex primer initiates RNA-templated DNA synthesis by the RT, resulting in the extension of the non-target DNA strand. Strand extension produces a 3′ flap structure containing the desired modifications. The edited flap competes for base pairing with the cognate target strand containing the original sequence, generating an equilibrium between a 3′ flap containing the edited sequence and a 5′ flap lacking the edited sequence (Figure 6B, step 1). The observation that prime editing can be applied successfully in both yeast and mammalian cells implies that the machinery that resolves prime editing intermediates is conserved among eukaryotes (Anzalone et al., 2019). Indeed, DNA flaps are structures frequently processed during DNA replication and repair. In particular, 5′ flap structures can be removed by the FEN1, EXO1, and DNA2 nucleases, while 3′ flaps are generally cleaved by endonucleases of the XPF family (Balakrishnan and Bambara, 2013; Ciccia et al., 2008; Sertic et al., 2020; Zheng et al., 2020). Given the equilibrium between 5′ and 3′ flaps in prime editing intermediates, editing outcomes vary depending on which flap is excised, such that 3′ edited flap cleavage restores the original genomic sequence, while 5′ flap excision leads to incorporation of the edited sequence (Figure 6B, steps 2 and 13). Consistently, knockdown of the 5′ flap endonuclease FEN1 reduces the frequency of intended edits in prime editing experiments (Chen et al., 2021b). Furthermore, given the presence of a nick on the edited strand, the MMR machinery is targeted to that DNA strand to promote EXO1-mediated degradation of the mismatch generated by prime editing and restore the original sequence (Figure 6B, steps 4 and 5). Thus, loss of MMR proteins or EXO1, or inhibition of the MMR pathway, stimulates the efficiency of prime editing (Chen et al., 2021b; Ferreira da Silva et al., 2021b). Installing silent mutations in the vicinity of the edit of interest can also increase prime editing efficiency by weakening the recognition of the edited mismatch by the MMR machinery (Chen et al., 2021b). Moreover, prime editing efficiency can be stimulated by introducing a nick on the non-edited strand with a second gRNA, which can favor EXO1-mediated degradation of the non-edited strand or promote its displacement and excision, accompanied by DNA resynthesis using the edited strand as a template (Figure 6B, steps 6–11). Alternatively, enhanced installment of precise insertions or deletions can also be achieved by using paired pegRNAs that generate complementary DNA sequences on both DNA strands, thus limiting the formation of DNA mismatches (Figures 7A and 7B) (Anzalone et al., 2021; Choi et al., 2021b; Jiang et al., 2021; Lin et al., 2021; Zhuang et al., 2022).

Prime editing applications

Prime editing is a highly versatile technology with multiple advantages over other genome editing approaches. Distinct from HDR, prime editing does not rely on DSB formation or synthetic exogenous DNA donor molecules to replace targeted genomic sequences. In addition, prime editors can edit the genomic region containing the PAM, allowing for the introduction of silent mutations to block potential multi-turnover nCas9 cleavage, a current limitation of base editors. Prime editing also enables the incorporation of a greater variety of desirable genomic changes relative to base editing. Although still in its early stages, highly versatile approaches and applications for

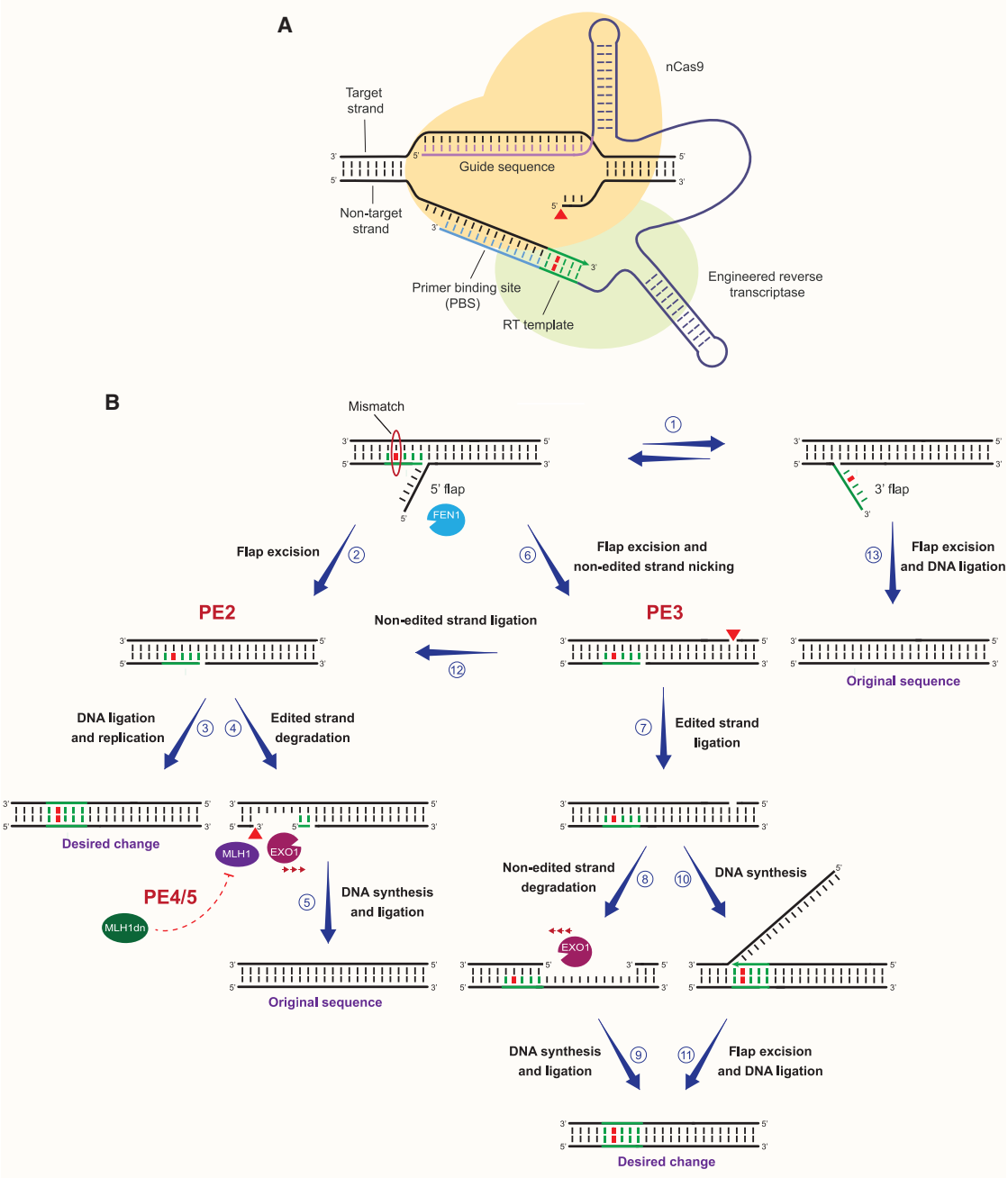

Figure 6. Repair of DNA lesions generated by prime editing

(A) Modules that constitute prime editors. Prime editors consist of the fusion of an engineered RT to nCas9. The RT utilizes an RNA:DNA heteroduplex formed upon the annealing of the nicked non-target DNA strand to a PBS in the pegRNA. The 3′ end of the nicked DNA strand is then extended by the RT using the pegRNA sequence containing the desired edits as a template (RT template).

(B) Processing of DNA intermediates generated by prime editors. Prime-editor-mediated reverse transcription leads to the generation of a 3′ flap containing the edit, in equilibrium with a 5′ flap that does not contain the edit (1). Formation of the 5′ flap is accompanied by the generation of heteroduplex DNA containing a mismatch between the edited strand and the parental strand (1). In PE2 prime editing systems, excision of the 5′ flap by FEN1 can be followed by resolution of the mismatch upon ligation of the nicked strand and DNA replication, leading to the incorporation of the desired change (2 and 3). Recognition of the mismatch by the MMR machinery can lead to MLH1- and EXO1-dependent degradation of the nicked edited strand, restoring the original sequence (4 and 5). In PE3 prime editing systems, introduction of a nick on the 5′ side of the mismatch using a second gRNA can engage the MMR machinery on the non-edited strand and promote its degradation by EXO1, resulting in the incorporation of the desired change following DNA synthesis (6–9). Incorporation of the edits of interest in PE3 systems can also occur upon DNA synthesis initiated by the 3′ end of the nicked non-edited strand, followed by strand displacement and excision of the resulting 5′ flap (10 and 11). Engagement of the MMR machinery on the nicked edited strand can lead to the restoration of the original sequence also in PE3 systems (12; 4 and 5). To suppress MMR-mediated restoration of the original sequence, the PE4 and PE5 prime editing systems combine respectively PE2- and PE3-based approaches with the transient expression of a dominant negative MLH1 protein (MLH1dn) (4). Excision of the 3′ flap containing the edited sequence results in MMR-independent restoration of the original sequence (13).

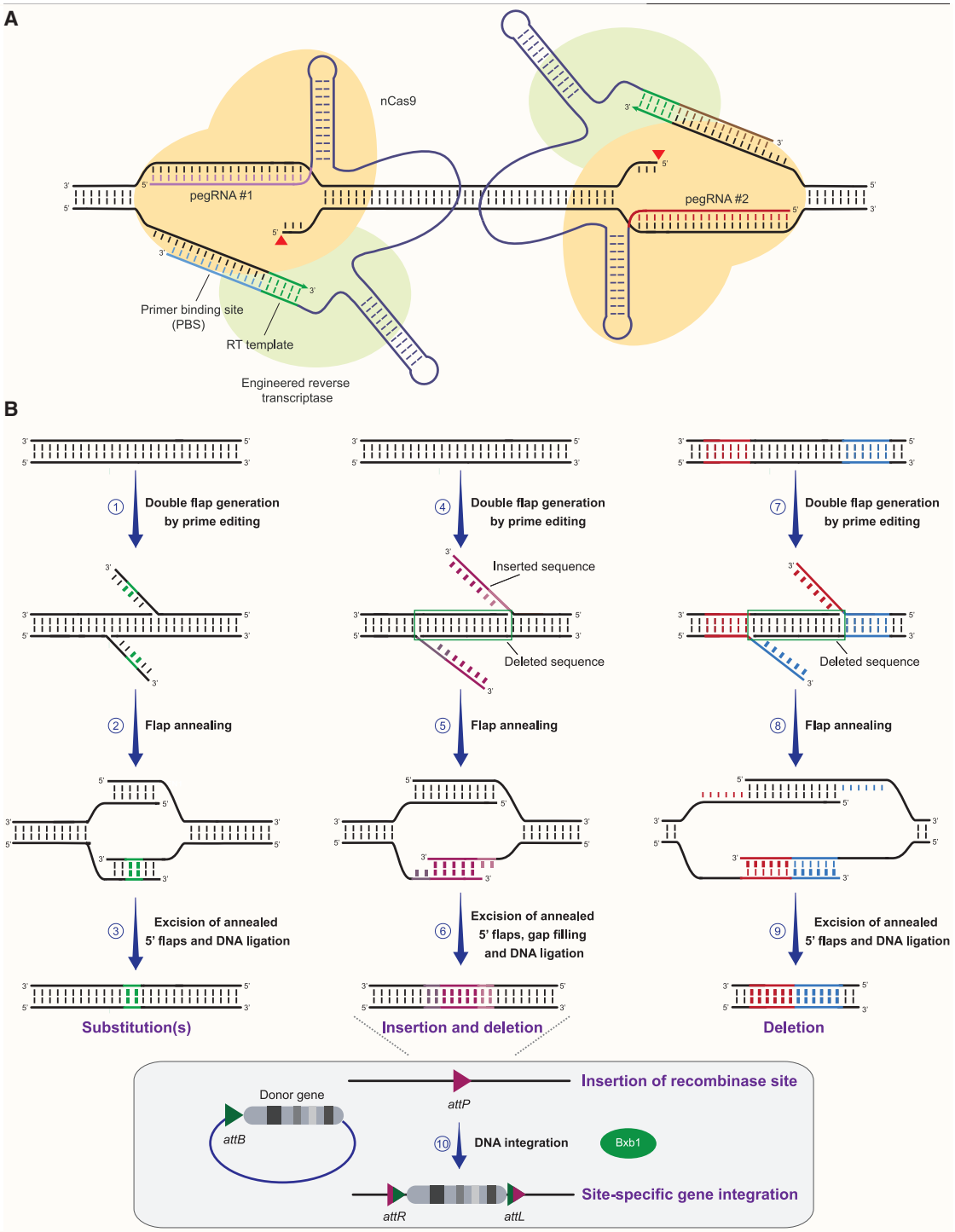

Figure 7. Processing of DNA intermediates induced by paired prime editors to generate precise substitutions, insertions, and/or deletions

(A) Design of paired prime editing strategies. A pair of prime editors is targeted to opposite DNA strands to generate two 3′ flaps with complementary sequences and desired modifications.

(B) Mechanisms for the generation of precise substitutions, insertions, and/or deletions using paired prime editors. Substitutions (left) can be obtained using paired prime editors that generate two complementary 3′ flaps containing the desired edit(s) (green) (1). Annealing of the two edited 3′ flaps and the two corresponding 5′ flaps containing the parental DNA sequence, followed by excision of the annealed 5′ flaps and ligation of the nicked strands, can then lead to the incorporation of the desired substitution(s) (2 and 3). Precise insertions and deletions (middle) can be induced using paired prime editors that generate two 3′ flaps that contain complementary (purple) and non-complementary (lighter and darker purple) sequences of the desired insert adjacent to the genomic sequence to be replaced (4). Annealing of the two edited 3′ flaps and the two parental 5′ flaps, followed by excision of the annealed 5′ flaps, filling of ssDNA gaps within the insert

(legend continued on next page)

prime editing have already been developed. Indeed, prime editing has been exploited to efficiently generate transition and transversion mutations, small insertions and deletions, and combinations of mutations in cancer and primary cells (Aida et al., 2020; Anzalone et al., 2019; Benamozig et al., 2021; Habib et al., 2021; Kweon et al., 2021; Liu et al., 2020b; Petri et al., 2021), patient-derived organoids (Schene et al., 2020), zebrafish (Petri et al., 2021), flies (Bosch et al., 2021), bacteria (Tong et al., 2021), and plants (Lin et al., 2020). Small genomic insertions can be introduced at higher efficiency, although with increased indel formation, using prime editors that generate DSBs and engage end joining pathways (Peterka et al., 2021). Moreover, prime editing has been utilized for saturation mutagenesis studies, allowing the interrogation of single genomic bases without introducing DSBs or editing byproducts (Erwood et al., 2021). Prime editing has also been used to develop genome-based systems that enable multiplex recording of cell lineage histories, transcriptional activities, and signaling events (Chen et al., 2021d; Choi et al., 2021a). Importantly, recent studies have demonstrated that prime editing can be used to correct pathogenic mutations in adult mice (Böck et al., 2021; Jang et al., 2021; Liu et al., 2021b), suggesting a promising future for this technology in therapeutic applications.

Limitations and current improvements of prime editing

The current main limitations of prime editing are its lower editing efficiency and more complex gRNA design relative to other precision genome editing technologies, such as base editing. However, recent reports in human cells and plants have shown that high-efficiency pegRNAs can be designed based on Cas9 activity prediction models and sequence contexts, highlighting, for example, that editing efficiency is influenced by the length of the PBS sequence and the RT template and by the GC base content and secondary structure of the insert sequence (Kim et al., 2021b; Koeppel et al., 2021; Lin et al., 2021). It is expected that prime editing will follow the same path of other genome editing technologies, such as base editing, leading to the design of more efficient prime editors. Such editors could be achieved, for example, by improving the processivity and fidelity of the RT, engineering the 3′ extension of the gRNA, developing machine learning algorithms to predict pegRNA efficiency in various cellular systems, and fusing, co-expressing, or inhibiting DNA repair factors to modulate prime editing outcome. Indeed, recent studies have already shown that prime editing efficiency can be enhanced by engineering pegRNAs and prime editors and modulating cellular DNA repair. For instance, adding hairpin structures to the 3′ end of the pegRNA, which contains the RT template sequence and the PBS, protects pegRNAs from degradation by cellular exonucleases and enhances prime editing efficiency (Chai et al., 2021; Liu et al., 2021c; Nelson et al., 2021). Prime editing events can also be enhanced by optimizing the codon sequence of the RT (Chen et al., 2021b), by increasing the localization of prime editors into the nucleus using multiple nuclear localization signal sequences (Chen et al., 2021b; Liu et al., 2021b), by introducing mutations in Cas9 that improve its nickase activity (Chen et al., 2021b), and by expressing a RT fusion that is recruited to prime editing sites through a MS2 RNA aptamer fused to the pegRNA (Chai et al., 2021). Furthermore, prime editing efficiency can be stimulated by co-expressing a dominant negative version of the MMR protein MLH1 to disrupt MMR-mediated degradation of the edited DNA bases (Chen et al., 2021b) or by fusing the DNA binding domain of RAD51 to prime editors to stabilize the pairing of the pegRNA to the nicked target DNA strand (Song et al., 2021). Prime editor fusions to small peptides derived from DNA repair proteins have also been shown to display greater editing efficiency, although their mechanisms of action still remain to be elucidated (Velimirovic et al., 2021). Recent studies have also reported improvement of prime editing efficiency through marker-free co-selection of edits simultaneously introduced in a gene of interest and *ATP1A1*, an essential gene encoding for the main subunit of the sodium/potassium pump (Levesque et al., 2021).

Prime editing strategies relying on dual nicking events (e.g., PE3, paired prime editors) can lead to the formation of DSBs (Figures 6B and 7B), resulting in increased frequency of indel byproducts relative to single-nick-based prime editing approaches (e.g., PE2) (Anzalone et al., 2019; Chen et al., 2021b; Lin et al., 2021). However, the frequency of indel byproducts, which is significantly lower than that obtained using Cas9 nucleases (Anzalone et al., 2019, 2021), can be reduced by inhibiting MMR (Chen et al., 2021b). Prime editing is highly specific, showing a low frequency of pegRNA-dependent off-target edits and no observable pegRNA-independent off-targets in plants and human cells (Gao et al., 2021a; Jin et al., 2021). Whole-genome sequencing of plants has also revealed that prime editors do not change retrotransposon copy number or telomere structure (Jin et al., 2021). While prime editing leads to minimal perturbations of the cellular transcriptome (Anzalone et al., 2019), the M-MLV RT utilized for prime editing has been shown to promote the readthrough of STOP codons through its interaction with the translation pre-termination complex (Tang et al., 2016; Zheng et al., 2021), thereby potentially affecting protein synthesis. However, this activity can be disrupted by deleting the RNase H domain of the M-MLV RT without resulting in a reduction of prime editing efficiency (Zheng et al., 2021). Given their smaller size, prime editors lacking the RNase H domain of the RT have been employed for *in vivo* editing following delivery by adeno-associated virus (AAV) vectors (Böck et al., 2021; Gao et al., 2021b).

RETRON-BASED GENOME EDITING

Retrons are bacterial genetic elements composed of a RT and a non-coding RNA that generate chimeric RNA-DNA molecules to

sequence, and DNA ligation, can lead to the replacement of the genomic sequence of interest with the desired insert (5 and 6). Deletions (right) can be obtained using paired prime editors that generate 3′ flaps (blue and red) complementary to genomic sequences upstream and downstream of the generated flaps (blue and red) (7). Annealing of the 3′ flaps with their complementary genomic sequences, followed by excision of the corresponding annealed 5′ flaps and ligation of the nicked strands, can result in the deletion of the genomic sequence located between the two 3′ flaps (8 and 9). Paired prime editors can be utilized for the insertion of serine recombinase sites (e.g., *attP* or *attB* sites), enabling site-specific integration of gene-size fragments upon transfection of donor gene constructs and expression of serine recombinases (e.g., Bxb1) (10). Site-specific integration can also be obtained using a prime editor fused to Bxb1 (not shown).

protect cells against phages (Millman et al., 2020). CRISPR-dependent retron systems encode a gRNA fused to a retron RNA, which is used as a template by a RT to produce a multi-copy ssDNA product tethered to the retron RNA (Simon et al., 2019). CRISPR/retron systems have been employed to generate ssDNA donors *in cellulo* to serve as templates for HDR-mediated introduction of targeted substitutions in the genomes of bacteria, yeast, and human cells (Lim et al., 2020; Sharon et al., 2018; Zhao et al., 2021a). In their current format, however, these systems rely on the generation of DSBs and provide limited HDR stimulation in human cells (Zhao et al., 2021a). DSB- and CRISPR-independent retron systems that rely on the recombineering of ssDNA products into the bacterial genome have recently been developed for high-throughput generation of nucleotide variants and other genome engineering applications (Farzadfard et al., 2021; Schubert et al., 2021). ssDNA-based recombineering approaches have also enabled the generation of mutant yeast strains at scale through RAD51-independent annealing of ssDNA oligos to the lagging strand of replication forks (Barbieri et al., 2017). However, whether CRISPR-independent retron systems can be employed to engineer eukaryotic cells remains to be determined.

INTEGRATION-BASED GENOME EDITING

CRISPR-associated recombinase systems

Insertion of large DNA sequences is critical for many biomedical applications, including gene therapy (High and Roncarolo, 2019). Site-specific insertion of genetic payloads can be achieved by HDR or NHEJ approaches. However, as discussed above, these methods suffer from the introduction of DSB intermediates and the generation of editing byproducts.

Site-specific recombinases are widely used as genetic tools for their ability to enable robust and stable DNA manipulations (Merrick et al., 2018; Olorunniji et al., 2016). To enhance their specificity, recombinases have been fused to genome editing effectors. The fusion of serine recombinase domains to programmable TALE and zinc-finger effectors and to dCas9 has resulted in site-directed insertion or deletion of desired DNA sequences at exogenous recombinase recognition sites or at endogenous genomic pseudo-sites recognized by recombinases, albeit with limited efficiency (Chaikind et al., 2016; Gersbach et al., 2011; Gordley et al., 2009; Mercer et al., 2012). Recent studies led to the development of improved CRISPR-based recombinase systems that employ prime editing to insert recognition sequences of site-specific recombinases (e.g., *attB* or *attP* sites) using single or paired pegRNAs, enabling targeted integration of gene-size payloads into the genome by serine recombinases (e.g., Bxb1) ectopically expressed or fused to prime editors (Anzalone et al., 2021; Ioannidi et al., 2021) (Figure 7B, step 10).

CRISPR-associated transposases

Transposases are self-contained enzymatic machineries that insert or excise DNA segments in the genome (Rebollo et al., 2012). Transposases act by recognizing and excising cognate donor sites located at the ends of the transposable element (Muñoz-López and García-Pérez, 2010). The transposable element is then integrated to new non-homologous insertion sites. Transposition requires multiple DNA repair activities. For instance, loss of host NHEJ repair factors affect piggyBat excision and repair of the broken donor backbone in yeast (She et al., 2015). Transposition results in the duplication of the transposon end sequences originating from the repair of ssDNA gaps generated during the integration of the transposon into the genome (Hickman and Dyda, 2016).

Recent computational analysis of bacterial genomes revealed the association of bacterial Tn7-like transposons with nuclease-defective CRISPR systems (Peters et al., 2017), suggesting the potential existence of RNA-guided transposition in bacteria (Faure et al., 2019). Subsequent studies successfully reconstituted minimal RNA-guided transposition using type I and type V CRISPR systems, resulting in site-specific insertion of large DNA fragments in bacteria (Klompe et al., 2019; Strecker et al., 2019b). These studies led to the characterization of insert transposable elements by guide RNA-assisted targeting (INTEGRATE) and CRISPR-associated transposase (CAST) systems for site-specific transposition of genetic cargos into the bacterial genome. While INTEGRATE is based on the CRISPR–Cas type I-F system, which lacks the Cas3 nuclease–helicase and is associated with the transposition protein TniQ, CAST employs a Tn7-like transposon associated with the CRISPR–Cas type V-K system, which contains a catalytically inactive Cas12k nuclease (Klompe et al., 2019; Strecker et al., 2019b). Comparative studies have shown that the INTEGRATE system is more efficient and displays higher purity of products relative to CAST (Rubin et al., 2021; Vo et al., 2021).

Alternative approaches led to the development of an engineered synthetic RNA-guided transposition system, called Cas-Transposon (Cas-Tn), by fusing the Himar1 transposase to dCas9 to induce site-specific transposition of DNA cargos into plasmids in bacteria (Chen and Wang, 2019). However, it remains to be determined whether the Cas-Tn system allows integration of cargos into the bacterial and eukaryotic genomes. More recent studies have reported the development of a transposition-based system that relies on the fusion of an engineered piggyBac transposase domain to Cas9 (Pallarès-Masmitjà et al., 2021). This system enables site-specific integration of multi-kilobase piggyBac transposon constructs into the mammalian genome (Pallarès-Masmitjà et al., 2021).

Applications and current limitations of CRISPR-associated integration-based systems

CRISPR-associated DNA integration systems generally operate without formation of DSBs and do not require the presence of DNA sequences homologous to the targeted genomic sequence in DNA donor molecules (Anzalone et al., 2021; Ioannidi et al., 2021; Klompe et al., 2019; Strecker et al., 2019b). While CRISPR-associated recombinase systems require prior insertion of exogenous sequences into the genome, CRISPR-based transposition enables integration of large DNA cargos at any genomic sequence of interest. However, CRISPR-based transposition is not scarless, resulting in the incorporation of transposon end sequences into the genome and the duplication of targeted genomic sites (Klompe et al., 2019; Strecker et al., 2019b). Further studies will be required to minimize the amount of undesired genetic changes induced by CRISPR-associated

Table 1. Strategies utilized to improve CRISPR-based HDR at Cas9-induced DSBs

Strategy	Proposed mechanism of action	Treatment	Treatment format	Delivery of Cas9	Donor type	Cell line	Effect	References
Optimizing the donor DNA	Improved annealing of the DNA donor to the asymmetrically released 3′ end of the non-target strand following Cas9 cleavage	Asymmetric ssODN	Oligonucleotide	RNP	ssODN	• HEK293	↑ HDR	Richardson et al., 2016
	Generation of DNA donor ends that facilitate HDR	dsDNA donor with 3′ overhangs	Plasmid, oligonucleotide	RNP	dsDNA	• HEK293 • mESCs • Mouse zygotes	↑ HDR	Hirotsune et al., 2020; Liang et al., 2017
	Chromatin-mediated stimulation of HDR	Chromatinized dsDNA donor	Plasmid	Plasmid	dsDNA	• MCF10A • HeLa	↑ HDR	Cruz-Becerra and Kadonaga, 2020
	Inhibition of DNA donor degradation	ssODN with phosphorothioate-modified ends	Oligonucleotide	Plasmid, mRNA	ssODN	• U2OS • RPE1 • Rat C6 • Rat, mouse, and zebrafish embryos	↑ HDR	Renaud et al., 2016
	Inhibition of DNA donor multimerization	dsDNA donor with biotin-modified 5′ ends	DNA amplicon	mRNA	dsDNA	• Medaka zygotes	↓ Illegitimate DNA donor integration	Gutierrez-Triana et al., 2018
	Enhanced DNA donor uptake	dsDNA or ssDNA/dsDNA hybrid donor with Cas9 target sequences	Oligonucleotide + RNP	RNP	dsDNA, ssDNA/dsDNA	• T cells • B cells • NK cells • hHSPCs	↑ HDR	Nguyen et al., 2020; Shy et al., 2021
	gRNA-mediated localization of the DNA donor to the Cas9-induced DSB	gRNA-ssODN covalently linked fusion	Oligonucleotide + RNP	RNP	ssODN	• HEK293	↑ HDR	Lee et al., 2017
		gRNA-S1m aptamer-streptavidin/biotin-ssODN complex	Oligonucleotide + RNP	RNP	ssODN	• hPSCs • HEK293	↑ HDR	Carlson-Stevermer et al., 2017
	Cas9-mediated localization of the DNA donor to the DSB	Cas9-avidin/biotin-ssODN complex	Oligonucleotide + RNP / plasmid / mRNA	RNP, plasmid, mRNA	ssODN	• HEK293 • Mouse zygotes	↑ HDR	Ma et al., 2017b
		ssODN covalently tethered to a Cas9-PCV fusion	Oligonucleotide + RNP	RNP	ssODN	• HEK293 • U2OS	↑ HDR	Aird et al., 2018
		ssODN covalently tethered to a Cas9-SNAP tag fusion	Oligonucleotide + RNP	RNP	ssODN	• HEK293 • K562 • mESCs	↑ HDR	Savic et al., 2018
		DBCO-ssODN covalently tethered to an azido-modified Cas9	Oligonucleotide + RNP	RNP	ssODN	• HEK293 • Mouse zygotes	↑ HDR	Ling et al., 2020

(*Continued on next page*)

Table 1. ***Continued***

Strategy	Proposed mechanism of action	Treatment	Treatment format	Delivery of Cas9	Donor type	Cell line	Effect	References
Controlling the cell cycle	G1/S phase synchronization: DNA polymerase α inhibition	Aphidicolin	Small molecule	RNP	ssODN	• HEK293 • Neonatal dermal fibroblasts	↑ HDR	Lin et al., 2014
	S phase synchronization: CDC7 inhibition	XL413	Small molecule	RNP	ssODN, dsDNA	• HEK293 • HeLa • U-251 • K562 • hiPSCs • Primary HSPCs • Primary T cells	↑ HDR ↓ NHEJ	Wienert et al., 2020
	G2/M phase synchronization: inhibition of microtubule polymerization	Nocodazole	Small molecule	RNP	ssODN, dsDNA	• HEK293	↑ HDR	Lin et al., 2014
		ABT-751	Small molecule	Plasmid	dsDNA	• hESCs • hiPSCs	↑ HDR	Yang et al., 2016
	G2/M phase synchronization: CDK1 inhibition	RO-3306	Small molecule	mRNA	AAV6	• hHSPCs	↑ HDR ↓ End joining	Lomova et al., 2019
Restricting Cas9 function during the cell cycle	Limiting Cas9 activity to the S/G2 phase	Cas9-geminin fusion	Plasmid	Plasmid	dsDNA	• HEK293	↑ HDR	Gutschner et al., 2016
			mRNA	mRNA	AAV6	• hHSPCs	↑ HDR ↓ NHEJ	Lomova et al., 2019
			Plasmid	Plasmid	dsDNA	• HEK293	↑ HDR	Charpentier et al., 2018
		Cas9 injection in the S phase	mRNA, RNP	mRNA, RNP	dsDNA	• Mouse zygotes	↑ HDR	Abe et al., 2020
		Cas9 injection in the G2 phase	mRNA	mRNA	dsDNA	• Mouse embryos	↑ HDR	Gu et al., 2018
Promoting open chromatin	Histone deacetylase (HDAC) inhibition, acetylation-mediated regulation of DSB repair factors	Trichostatin A (TSA), PCI-24781	Small molecule	Plasmid, RNP	dsDNA, ssODN	• Pig fetal fibroblasts • Pig embryos	↑ HDR, MMEJ	Li et al., 2020b
	HDAC inhibition	TSA	Small molecule	Cas9-expressing cell line	None	• HepG2	↑ End joining	Chakrabarti et al., 2019
		Entinostat, Panobinostat	Small molecule	Plasmid, AdV	dsDNA	• HEK293 • HeLa • HT29	↑ HDR, End joining	Liu et al., 2020a
		Valproic acid	Small molecule	Plasmid	dsDNA	• hESCs • hiPSCs	↑ HDR	Takayama et al., 2017

(*Continued on next page*)

Table 1. ***Continued***

Strategy	Proposed mechanism of action	Treatment	Treatment format	Delivery of Cas9	Donor type	Cell line	Effect	References
Inhibiting NHEJ	Ku depletion	shRNA, siRNA	Plasmid, siRNA	Plasmid	dsDNA	• HEK293	↑ HDR ↓ NHEJ	Chu et al., 2015; Robert et al., 2015
	LIG4 inhibition	shRNA, siRNA	Plasmid, siRNA	Plasmid	dsDNA	• HEK293	↑ HDR ↓ NHEJ	Chu et al., 2015; Robert et al., 2015
		SCR7	Small molecule	Cas9-expressing cell lines, mRNA	dsDNA, ssODN	• A549 • MelJuSo • mDC2.4 • Mouse zygotes	↑ HDR ↓ NHEJ	Maruyama et al., 2015
				Plasmid	dsDNA	• HEK293	↑ HDR ↓ NHEJ	Chu et al., 2015; Pinder et al., 2015; Robert et al., 2015
				Plasmid, Cas9-expressing cell lines	dsDNA, ssODN	• HEK293 • hiPSCs	No HDR stimulation	Gutschner et al., 2016; Riesenberg and Maricic, 2018; Zhang et al., 2017
		Expression of the Ad4/Ad5 proteins E1B55K and E4orf6	Plasmid	Plasmid	dsDNA	• HEK293 • Mouse Burkitt lymphoma cells	↑ HDR ↓ NHEJ	Chu et al., 2015; Robert et al., 2015
	DNA-PKcs inhibition	siRNA	siRNA	Plasmid	dsDNA	• HEK293	↑ HDR ↓ NHEJ	Robert et al., 2015
		NU7441	Small molecule	Plasmid	dsDNA, ssODN	• HEK293 • MEFs	↑ HDR ↓ NHEJ	Robert et al., 2015
				Plasmid	dsDNA	• hiPSC	↑ HDR	Zhang et al., 2017
				RNP	None	• HEK293	↑ MMEJ ↓ NHEJ	van Overbeek et al., 2016
				Plasmid	dsDNA	• U2OS	↑ HDR	Canny et al., 2018
		NU7026	Small molecule	Plasmid	dsDNA	• HEK293	↑ HDR ↓ NHEJ	Suzuki et al., 2016
		KU-0060648 (also inhibits PI3K	Small molecule	Plasmid	dsDNA, ssODN	• HEK293 • MEFs	↑ HDR ↓ NHEJ	Robert et al., 2015
		M3814	Small molecule	RNP	ssODN	• K562 • hiPSCs	↑ HDR ↓ End joining	Riesenberg et al., 2019

(*Continued on next page*)

CellPress

Table 1. ***Continued***

Strategy	Proposed mechanism of action	Treatment	Treatment format	Delivery of Cas9	Donor type	Cell line	Effect	References
Promoting end resection	Inhibition of 53BP1	i53 (inhibitor of 53BP1	Plasmid, AAV	Plasmid, RNP	ssODN, dsDNA	• U2OS • HEK293 • K562 • MCF10A • MEFs	↑ HDR	Canny et al., 2018
		e18 (enhanced RAD18 variant	Plasmid, mRNA	Plasmid, mRNA	ssODN, dsDNA	• HEK293 • HeLa • U2OS • hESCs	↑ HDR, MMEJ ↓ NHEJ	Nambiar et al., 2019
		Cas9-DN1S (dominant negative 53BP1 mutant) fusion	Plasmid, RNP	Plasmid, RNP	dsDNA, scAAV, ssAAV	• HEK293 • K562 • LCL • Jurkat • Patient-derived B lymphocytes	↑ HDR ↓ NHEJ	Jayavaradhan et al., 2019
	Engagement of CtIP	Cas9-HE (minimal N-terminal CtIP fragment) fusion	Plasmid, mRNA	Plasmid, mRNA	dsDNA	• HEK293 • HCT116 • RG37 • hiPSCs • Rat oocytes	↑ HDR, MMEJ ↓ NHEJ	Charpentier et al., 2018
	Engagement of EXO1	Cas9-HR (EXO1 fragment) fusion	Plasmid	Plasmid	dsDNA	• A549 • H1299 • K562	↑ HDR	Hackley, 2021
Inhibiting MMEJ	POLQ inactivation	*POLQ* knockout	Cas9-mediated gene knockout	Plasmid	None	• miPSCs	↓ MMEJ	Mateos-Gomez et al., 2015
					dsDNA	• MEFs • mESCs	↑ HDR	Mateos-Gomez et al., 2017
Promoting strand annealing	RAD52 stimulation	RAD52 overexpression	Plasmid	Plasmid	ssODN	• hiPSCs • HEK293	↑ HDR	Paulsen et al., 2017
		Cas9-Rad52 (yeast homolog) fusion	Plasmid	Plasmid	dsDNA, ssODN	• HEK293 • PK15	↑ HDR	Shao et al., 2017
Promoting DNA recombination	RAD51 stimulation	RS-1	Small molecule	Plasmid	dsDNA	• HEK293 • U2OS	↑ HDR	Pinder et al., 2015
		RAD51 overexpression	mRNA	mRNA	dsDNA	• Rabbit embryos	↑ HDR	Song et al., 2016
	RAD51 localization	Expression of miCas9 (fusion of BRCA2 exon 27 to SpCas9	Plasmid, RNP	Plasmid, RNP	ssODN, dsDNA	• Human fibroblasts • AECs • hiPSCs • Ad293 • hHSCs • Jurkat	↑ HDR ↓ End joining	Ma et al., 2020
	BRCA1 modulation	Expression of the BRCA1 variant BRCA1^{M1775R}	Plasmid	Plasmid	dsDNA	• HEK293	↑ HDR	Pinder et al., 2015

(*Continued on next page*)

Table 1. *Continued*

Strategy	Proposed mechanism of action	Treatment	Treatment format	Delivery of Cas9	Donor type	Cell line	Effect	References
Enhancing DSB repair	Stimulation of DSB repair events	Cas9-POLD3 fusion	Plasmid, mRNA, RNP	Plasmid, mRNA, RNP	ssODN	• HEK293 • RPE1 • BJ • PBMCs • hESCs	↑ HDR, NHEJ	Reint et al., 2021
Combining multiple HDR regulators	Stimulation of G1 to S phase transition and G/M phase synchronization	Cyclin D1 overexpression + nocodazole treatment	Plasmid + small molecule	Plasmid	dsDNA	• hiPSCs	↑ HDR	Zhang et al., 2017
	G2/M phase synchronization followed by G1/S phase synchronization	Nocodazole + aphidicolin treatment	Small molecule	RNP	ssODN	• hESCs	↑ HDR	Lin et al., 2014
	G2/M phase synchronization + degradation of Cas9 in the G1 phase	RO-3306 + Cas9-geminin fusion	Small molecule + mRNA	mRNA	AAV6	• hHSPCs	↑ HDR ↓ End joining	Lomova et al., 2019
	Confinement of Cas9 activity to the G2 phase + localization of the DNA donor to the Cas9-induced DSB	Injection of a Cas9-streptavidin fusion and a biotin-dsDNA donor in the G2 phase	mRNA + DNA amplicon	mRNA	dsDNA	• Mouse embryos	↑ HDR	Gu et al., 2018
	Promotion of strand annealing and end resection	Expression of RAD52 and dominant-negative 53BP1 (dn53BP1	Plasmid	Plasmid	ssODN	• hiPSCs • HEK293	↑ HDR	Paulsen et al., 2017
	Inhibition of MMEJ and NHEJ	Disruption of POLQ and Ku	Cas9-mediated gene knockout	Plasmid	dsDNA	• mESCs	↓ Illegitimate DNA donor integration	Zelensky et al., 2017
	Modulation of multiple DSB repair proteins	CRISPY mix (NU7026 + TSA + MLN4924 + NSC 15520	Small molecule	nCas9-expressing cell line, plasmid	ssODN	• hiPSCs • hESCs	↑ HDR ↓ End joining	Riesenberg and Maricic, 2018
	End resection initiation and extension	eRAD18 (e18)-Cas9-CtIP fusion	Plasmid	Plasmid	dsDNA	• HEK293	↑ HDR	Richardson et al., 2020
	Cell cycle modulation + inhibition of NHEJ	XLF413 + cold shock + NU7441 + SCR7	Small molecule + temperature change	RNP	ssODN	• hiPSCs	↑ HDR ↓ End joining	Maurissen and Woltjen, 2020
	Promotion of end resection + inhibition of NHEJ	CRISPRa/i-mediated activation of CDK1 and repression of Ku80	Plasmid, lentivirus	Cas9-expressing cell lines	dsDNA	• HEK293 • HeLa	↑ HDR	Ye et al., 2018
	Stimulation of RAD51-mediated strand invasion + chromatin opening at target site	RAD51 overexpression + valproic acid treatment	Plasmid + small molecule	Plasmid	dsDNA	• hESCs • hiPSCs	↑ HDR	Takayama et al., 2017

(*Continued on next page*)

Table 1. ***Continued***

Strategy	Proposed mechanism of action	Treatment	Treatment format	Delivery of Cas9	Donor type	Cell line	Effect	References
Limiting cell toxicity associated with DSB formation and/or donor DNA delivery	Transient inhibition of p53	Expression of a dominant-negative p53 truncated form (GSE56	mRNA	RNP	AAV6	• hHSPCs	↑ HDR	Schiroli et al., 2019
	Transient inhibition of p53 + cell cycle modulation	Expression of the Ad5 protein E4orf6/7 and the dominant-negative p53 mutant protein GSE56	mRNA	RNP	AAV6, IDLV	• hHSPCs	↑ HDR, End joining	Ferrari et al., 2020
	Transient inhibition of p53	Overexpression of MDM2 (p53 antagonist	Plasmid	RNP	ssODN	• RPE1	↑ HDR	Haapaniemi et al., 2018
	Inhibition of apoptotic cell death	Overexpression of BCL-XL	Plasmid, lentivirus	Plasmid	dsDNA, ssODN	• hiPSCs • mESCs	↑ HDR, NHEJ	Li et al., 2018c
Of unknown mechanism	Unknown for genome editing; β3-adrenergic receptor activation	L755507	Small molecule	Plasmid	dsDNA, ssODN	• mESCs • HeLa • K562 • hiPSCs • Neural stem cells • HUVECs • CRL-2097	↑ HDR ↓ End joining	Yu et al., 2015
	Unknown for genome editing; ER-Golgi transport inhibition	Brefeldin A	Small molecule	Plasmid	dsDNA	• mESCs	↑ HDR	
	Unknown; potential contribution of cell cycle-dependent and -independent effects	Cold shock (32°C for 1–2 days	Temperature change	mRNA	ssODN	• HEK293 • hiPSCs	↑ HDR	Guo et al., 2018
	Unknown	SHROOM1 depletion / deletion	siRNA, Cas9-mediated gene knockout	Plasmid, mRNA	dsDNA, ssODN	• HEK293T • HCT116 • Hepa1-6 • Mouse embryos	↑ HDR	Zhao et al., 2020
	Unknown	Resveratrol	Small molecule	Plasmid	dsDNA	• Porcine fetal fibroblasts	↑ HDR	Li et al., 2017

The mechanisms of action for each strategy are indicated, along with the type of treatment, the delivery method for Cas9, the DNA donor type, the cell lines utilized, and the effect observed on DSB repair. References of the studies that employ the indicated strategies are also listed. AAV, adeno-associated virus; AAV6, adeno-associated virus type 6; AdV, adenovirus; Ad4, adenovirus type 4; Ad5, adenovirus type 5; AECs, alveolar epithelial cells; CRISPRa/i, CRISPR activation and interference; DBCO, dibenzylcyclooctyne; hESCs, human embryonic stem cells; hHSCs, human hematopoietic stem cells; hHSPCs, human hematopoietic stem and progenitor cells; hiPSCs, human induced pluripotent stem cells; hPSCs, human pluripotent stem cells; HUVECs, human umbilical vein endothelial cells; IDLV, integrase defective lentiviral vector; LCL, lymphoblastoid B cell line, EBV-transformed; MEFs, mouse embryonic fibroblasts; mESCs, mouse embryonic stem cells; miPSCs, mouse induced pluripotent stem cells; PBMCs, peripheral blood mononuclear cells; PCV, porcine circovirus 2 Rep protein; RNP, ribonucleoprotein; scAAV, self-complementary adeno-associated virus; ssAAV, single-stranded adeno-associated virus.

transposition. Improved CRISPR-associated transposon systems will surely emerge upon further characterization of their structural features and molecular mechanisms (Halpin-Healy et al., 2020; Park et al., 2021; Petassi et al., 2020; Querques et al., 2021; Saito et al., 2021; Shen et al., 2021; Vo et al., 2021; Xiao et al., 2021). RNA-guided transposases have been exploited for high-throughput gene disruption studies in bacteria (Chen et al., 2021e) and for locus- and species-specific genetic manipulation of bacteria in complex microbial communities (Rubin et al., 2021; Vo et al., 2021). This approach has the potential to revolutionize the manipulation of bacterial communities in human-health-related environments, such as the gut microbiome, and symbiotic agricultural microbial communities. Additional work is, however, necessary to achieve programmable transposition using these approaches in mammalian cells. PiggyBac transposase-based CRISPR systems have been shown to promote site-specific integration of transgenes in mammalian cell lines and in the mouse liver (Pallarès-Masmitjà et al., 2021). However, these systems result in indel formation due their reliance on Cas9-induced DSBs, which stimulate the insertional activity of the piggyBac transposase (Pallarès-Masmitjà et al., 2021).

Prime-editing-dependent recombinase systems have been shown to enable efficient and site-specific integration of large DNA cargos into the genome of mammalian cells (Anzalone et al., 2021; Ioannidi et al., 2021). These approaches have been employed for inserting fluorescent markers and antibiotic resistance gene cassettes, labeling proteins with fluorescent tags, producing and secreting therapeutically relevant enzymes, and correcting pathogenic genomic inversions (Anzalone et al., 2021; Ioannidi et al., 2021). The recent discovery of a multitude of microbial serine recombinases with diverse target sites (Durrant et al., 2021) should provide enhanced efficiency, flexibility, and multiplexing capabilities to recombinase-based systems. Altogether, technologies relying on CRISPR-associated DNA integration hold great promise for clinical applications requiring the integration of fully functional therapeutic genes, the replacement of gene exons, or the insertion of engineered constructs, such as chimeric antigen receptors, in safe harbor loci while minimizing the safety concerns associated with the random insertion of viral constructs, as in current gene therapy strategies.

CONCLUSIONS AND FUTURE PERSPECTIVES

Programmable technologies enabling the introduction of site-specific DSBs have sparked a revolution in genome editing. In particular, the repurposing of natural CRISPR systems into powerful genome editing technologies and the development of new tools for genome engineering are transforming biology and medicine (Doudna, 2020; Kulkarni et al., 2021; Pickar-Oliver and Gersbach, 2019) and offering encouraging perspectives for the treatment of debilitating human diseases (Frangoul et al., 2021; Gillmore et al., 2021; Lu et al., 2020; Newby and Liu, 2021; Porteus, 2019; Stadtmauer et al., 2020; Xu et al., 2019). Moreover, DSB-free technologies, such as CRISPR-dependent base editing, prime editing, and DNA integration, have allowed unprecedented interrogation of genomes with high precision at single-base resolution and enabled highly efficient insertion of DNA constructs at sites of interest. These technologies hold great promise for the correction of disease-causing mutations and the development of mutation-specific or gene replacement therapies. The next generation of precision genome editing tools are likely to result from fundamental discoveries obtained by studying the wide diversity of prokaryotic organisms and the broad variety of anti-phage immune mechanisms (Hampton et al., 2020; Hug et al., 2016). For instance, recent studies have discovered several novel bacterial defense systems with unique properties and activities that might bolster the development of future genome engineering technologies (Altae-Tran et al., 2021; Doron et al., 2018; Gao et al., 2020; Karvelis et al., 2021; Rousset et al., 2021). These tools will help us define the fundamentals of organismal life, combat environmental pollution, produce improved food, and understand and treat genetic and infectious human diseases.

ACKNOWLEDGMENTS

We thank Richard Baer, Alex Chavez, David Liu, Lorraine Symington, and members of the Billon and Ciccia laboratories for insightful discussions and comments on the manuscript. We apologize to our colleagues whose work could not be cited because of space constraints. This work was supported by the generous start-up package from the University of Calgary, Cumming School of Medicine, the Arnie Charbonneau Cancer Institute, and the Robson DNA Science Centre to P.B. and NIH grants R01CA197774, R01CA227450, and P01CA174653 to A.C.

AUTHOR CONTRIBUTIONS

T.S.N., L.B., P.B., and A.C. wrote the text and prepared the figures. All authors approved the manuscript.

DECLARATION OF INTERESTS

The authors have filed a patent application related to the development of a new method for the detection of precision genome editing.

REFERENCES

Abe, T., Inoue, K.I., Furuta, Y., and Kiyonari, H. (2020). Pronuclear Microinjection during S-Phase Increases the Efficiency of CRISPR-Cas9-Assisted Knockin of Large DNA Donors in Mouse Zygotes. Cell Rep. *31*, 107653.

Abudayyeh, O.O., Gootenberg, J.S., Konermann, S., Joung, J., Slaymaker, I.M., Cox, D.B., Shmakov, S., Makarova, K.S., Semenova, E., Minakhin, L., et al. (2016). C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science *353*, aaf5573.

Abudayyeh, O.O., Gootenberg, J.S., Essletzbichler, P., Han, S., Joung, J., Belanto, J.J., Verdine, V., Cox, D.B.T., Kellner, M.J., Regev, A., et al. (2017). RNA targeting with CRISPR-Cas13. Nature *550*, 280–284.

Adikusuma, F., Williams, N., Grutzner, F., Hughes, J., and Thomas, P. (2017). Targeted Deletion of an Entire Chromosome Using CRISPR/Cas9. Mol. Ther. *25*, 1736–1738.

Adikusuma, F., Piltz, S., Corbett, M.A., Turvey, M., McColl, S.R., Helbig, K.J., Beard, M.R., Hughes, J., Pomerantz, R.T., and Thomas, P.Q. (2018). Large deletions induced by Cas9 cleavage. Nature *560*, E8–E9.

Aguirre, A.J., Meyers, R.M., Weir, B.A., Vazquez, F., Zhang, C.Z., Ben-David, U., Cook, A., Ha, G., Harrington, W.F., Doshi, M.B., et al. (2016). Genomic Copy Number Dictates a Gene-Independent Cell Response to CRISPR/Cas9 Targeting. Cancer Discov. *6*, 914–929.

Aida, T., Wilde, J.J., Yang, L., Hou, Y., Li, M., Xu, D., Lin, J., Qi, P., Lu, Z., and Feng, G. (2020). Prime editing primarily induces undesired outcomes in mice. bioRxiv. https://doi.org/10.1101/2020.08.06.239723.

Aird, E.J., Lovendahl, K.N., St Martin, A., Harris, R.S., and Gordon, W.R. (2018). Increasing Cas9-mediated homology-directed repair efficiency through covalent tethering of DNA repair template. Commun Biol. *1*, 54.

Al-Zain, A.M., and Symington, L.S. (2021). The dark side of homology-directed repair. DNA Repair (Amst.) *106*, 103181.

Alanis-Lobato, G., Zohren, J., McCarthy, A., Fogarty, N.M.E., Kubikova, N., Hardman, E., Greco, M., Wells, D., Turner, J.M.A., and Niakan, K.K. (2021). Frequent loss of heterozygosity in CRISPR-Cas9-edited early human embryos. Proc. Natl. Acad. Sci. USA *118*, e2004832117.

Allen, F., Crepaldi, L., Alsinet, C., Strong, A.J., Kleshchevnikov, V., De Angeli, P., Páleníková, P., Khodak, A., Kiselev, V., Kosicki, M., et al. (2019). Predicting the mutations generated by repair of Cas9-induced double-strand breaks. Nat. Biotechnol. *37*, 64–72.

Alseth, I., Dalhus, B., and Bjørås, M. (2014). Inosine in DNA and RNA. Curr. Opin. Genet. Dev. *26*, 116–123.

Altae-Tran, H., Kannan, S., Demircioglu, F.E., Oshiro, R., Nety, S.P., McKay, L.J., Dlakić, M., Inskeep, W.P., Makarova, K.S., Macrae, R.K., et al. (2021). The widespread IS200/IS605 transposon family encodes diverse programmable RNA-guided endonucleases. Science *374*, 57–65.

Amoasii, L., Long, C., Li, H., Mireault, A.A., Shelton, J.M., Sanchez-Ortiz, E., McAnally, J.R., Bhattacharyya, S., Schmidt, F., Grimm, D., et al. (2017). Single-cut genome editing restores dystrophin expression in a new mouse model of muscular dystrophy. Sci. Transl. Med. *9*, eaan8081.

Amoasii, L., Hildyard, J.C.W., Li, H., Sanchez-Ortiz, E., Mireault, A., Caballero, D., Harron, R., Stathopoulou, T.R., Massey, C., Shelton, J.M., et al. (2018). Gene editing restores dystrophin expression in a canine model of Duchenne muscular dystrophy. Science *362*, 86–91.

Anand, R., Ranjha, L., Cannavo, E., and Cejka, P. (2016). Phosphorylated CtIP Functions as a Co-factor of the MRE11-RAD50-NBS1 Endonuclease in DNA End Resection. Mol. Cell *64*, 940–950.

Annunziato, S., Lutz, C., Henneman, L., Bhin, J., Wong, K., Siteur, B., van Gerwen, B., de Korte-Grimmerink, R., Zafra, M.P., Schatoff, E.M., et al. (2020). In situ CRISPR-Cas9 base editing for the development of genetically engineered mouse models of breast cancer. EMBO J. *39*, e102169.

Anzalone, A.V., Randolph, P.B., Davis, J.R., Sousa, A.A., Koblan, L.W., Levy, J.M., Chen, P.J., Wilson, C., Newby, G.A., Raguram, A., and Liu, D.R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. Nature *576*, 149–157.

Anzalone, A.V., Koblan, L.W., and Liu, D.R. (2020). Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime. Nat. Biotechnol. *38*, 824–844.

Anzalone, A.V., Gao, X.D., Podracky, C.J., Nelson, A.T., Koblan, L.W., Raguram, A., Levy, J.M., Mercer, J.A.M., and Liu, D.R. (2021). Programmable deletion, replacement, integration and inversion of large DNA sequences with twin prime editing. Nat. Biotechnol. Published online December 9, 2021. https://doi.org/10.1038/s41587-021-01133-w.

Arbab, M., Shen, M.W., Mok, B., Wilson, C., Matuszek, Ż., Cassa, C.A., and Liu, D.R. (2020). Determinants of Base Editing Outcomes from Target Library Analysis and Machine Learning. Cell *182*, 463–480.e30.

Artegiani, B., Hendriks, D., Beumer, J., Kok, R., Zheng, X., Joore, I., Chuva de Sousa Lopes, S., van Zon, J., Tans, S., and Clevers, H. (2020). Fast and efficient generation of knock-in human organoids using homology-independent CRISPR-Cas9 precision genome editing. Nat. Cell Biol. *22*, 321–331.

Balakrishnan, L., and Bambara, R.A. (2013). Flap endonuclease 1. Annu. Rev. Biochem. *82*, 119–138.

Barbieri, E.M., Muir, P., Akhuetie-Oni, B.O., Yellman, C.M., and Isaacs, F.J. (2017). Precise Editing at DNA Replication Forks Enables Multiplex Genome Engineering in Eukaryotes. Cell *171*, 1453–1467.e13.

Barnes, D.E., and Lindahl, T. (2004). Repair and genetic consequences of endogenous DNA base damage in mammalian cells. Annu. Rev. Genet. *38*, 445–476.

Bashir, S., Dang, T., Rossius, J., Wolf, J., and Kühn, R. (2020). Enhancement of CRISPR-Cas9 induced precise gene editing by targeting histone H2A-K15 ubiquitination. BMC Biotechnol. *20*, 57.

Beard, W.A., Horton, J.K., Prasad, R., and Wilson, S.H. (2019). Eukaryotic Base Excision Repair: New Approaches Shine Light on Mechanism. Annu. Rev. Biochem. *88*, 137–162.

Benamozig, O., Baudrier, L., and Billon, P. (2021). Chapter Twelve - A detection method for the capture of genomic signatures: From disease diagnosis to genome editing. In Methods in Enzymology, B.F. Eichman, ed. (Academic Press), pp. 251–282.

Bermudez-Cabrera, H.C., Culbertson, S., Barkal, S., Holmes, B., Shen, M.W., Zhang, S., Gifford, D.K., and Sherwood, R.I. (2021). Small molecule inhibition of ATM kinase increases CRISPR-Cas9 1-bp insertion frequency. Nat. Commun. *12*, 5111.

Bhargava, R., Onyango, D.O., and Stark, J.M. (2016). Regulation of Single-Strand Annealing and its Role in Genome Maintenance. Trends Genet. *32*, 566–575.

Bibikova, M., Beumer, K., Trautman, J.K., and Carroll, D. (2003). Enhancing gene targeting with designed zinc finger nucleases. Science *300*, 764.

Bieging, K.T., Mello, S.S., and Attardi, L.D. (2014). Unravelling mechanisms of p53-mediated tumour suppression. Nat. Rev. Cancer *14*, 359–370.

Billon, P., Bryant, E.E., Joseph, S.A., Nambiar, T.S., Hayward, S.B., Rothstein, R., and Ciccia, A. (2017). CRISPR-Mediated Base Editing Enables Efficient Disruption of Eukaryotic Genes through Induction of STOP Codons. Mol. Cell *67*, 1068–1079.e4.

Billon, P., Nambiar, T.S., Hayward, S.B., Zafra, M.P., Schatoff, E.M., Oshima, K., Dunbar, A., Breinig, M., Park, Y.C., Ryu, H.S., et al. (2020). Detection of Marker-Free Precision Genome Editing and Genetic Variation through the Capture of Genomic Signatures. Cell Rep. *30*, 3280–3295.e6.

Boch, J., Scholze, H., Schornack, S., Landgraf, A., Hahn, S., Kay, S., Lahaye, T., Nickstadt, A., and Bonas, U. (2009). Breaking the code of DNA binding specificity of TAL-type III effectors. Science *326*, 1509–1512.

Böck, D., Rothgangl, T., Villiger, L., Schmidheini, L., Mathis, N., Ioannidi, E., Kreutzer, S., Kontarakis, Z., Rimann, N., Grisch-Chan, H.M., et al. (2021). Treatment of a metabolic liver disease by in vivo prime editing in mice. bioRxiv,. https://doi.org/10.1101/2021.08.17.456632.

Boel, A., De Saffel, H., Steyaert, W., Callewaert, B., De Paepe, A., Coucke, P.J., and Willaert, A. (2018). CRISPR/Cas9-mediated homology-directed repair by ssODNs in zebrafish induces complex mutational patterns resulting from genomic integration of repair-template fragments. Dis. Model. Mech. *11*, dmm035352.

Bosch, J.A., Birchak, G., and Perrimon, N. (2021). Precise genome engineering in *Drosophila* using prime editing. Proc. Natl. Acad. Sci. USA *118*, e2021996118.

Bothmer, A., Phadke, T., Barrera, L.A., Margulies, C.M., Lee, C.S., Buquicchio, F., Moss, S., Abdulkerim, H.S., Selleck, W., Jayaram, H., et al. (2017). Characterization of the interplay between DNA repair and CRISPR/Cas9-induced DNA lesions at an endogenous locus. Nat. Commun. *8*, 13905.

Botuyan, M.V., Lee, J., Ward, I.M., Kim, J.E., Thompson, J.R., Chen, J., and Mer, G. (2006). Structural basis for the methylation state-specific recognition of histone H4-K20 by 53BP1 and Crb2 in DNA repair. Cell *127*, 1361–1373.

Brambati, A., Barry, R.M., and Sfeir, A. (2020). DNA polymerase theta (Polθ) - an error-prone polymerase necessary for genome stability. Curr. Opin. Genet. Dev. *60*, 119–126.

Brinkman, E.K., Chen, T., de Haas, M., Holland, H.A., Akhtar, W., and van Steensel, B. (2018). Kinetics and Fidelity of the Repair of Cas9-Induced Double-Strand DNA Breaks. Mol. Cell *70*, 801–813.e6.

Brouns, S.J., Jore, M.M., Lundgren, M., Westra, E.R., Slijkhuis, R.J., Snijders, A.P., Dickman, M.J., Makarova, K.S., Koonin, E.V., and van der Oost, J. (2008). Small CRISPR RNAs guide antiviral defense in prokaryotes. Science *321*, 960–964.

Brunet, E., and Jasin, M. (2018). Induction of Chromosomal Translocations with CRISPR-Cas9 and Other Nucleases: Understanding the Repair Mechanisms That Give Rise to Translocations. Adv. Exp. Med. Biol. *1044*, 15–25.

Buisson, R., Langenbucher, A., Bowen, D., Kwan, E.E., Benes, C.H., Zou, L., and Lawrence, M.S. (2019). Passenger hotspot mutations in cancer driven by APOBEC3A and mesoscale genomic features. Science *364*, eaaw2872.

Burgio, G., and Teboul, L. (2020). Anticipating and Identifying Collateral Damage in Genome Editing. Trends Genet. *36*, 905–914.

Burns, M.B., Temiz, N.A., and Harris, R.S. (2013). Evidence for APOBEC3B mutagenesis in multiple human cancers. Nat. Genet. *45*, 977–983.

Burstein, D., Harrington, L.B., Strutt, S.C., Probst, A.J., Anantharaman, K., Thomas, B.C., Doudna, J.A., and Banfield, J.F. (2017). New CRISPR-Cas systems from uncultivated microbes. Nature *542*, 237–241.

Caldecott, K.W. (2014). DNA single-strand break repair. Exp. Cell Res. *329*, 2–8.

Caldecott, K.W. (2020). Mammalian DNA base excision repair: Dancing in the moonlight. DNA Repair (Amst.) *93*, 102921.

Cameron, P., Coons, M.M., Klompe, S.E., Lied, A.M., Smith, S.C., Vidal, B., Donohoue, P.D., Rotstein, T., Kohrs, B.W., Nyer, D.B., et al. (2019). Harnessing type I CRISPR-Cas systems for genome engineering in human cells. Nat. Biotechnol. *37*, 1471–1477.

Campa, C.C., Weisbach, N.R., Santinha, A.J., Incarnato, D., and Platt, R.J. (2019). Multiplexed genome engineering by Cas12a and CRISPR arrays encoded on single transcripts. Nat. Methods *16*, 887–893.

Canny, M.D., Moatti, N., Wan, L.C.K., Fradet-Turcotte, A., Krasner, D., Mateos-Gomez, P.A., Zimmermann, M., Orthwein, A., Juang, Y.C., Zhang, W., et al. (2018). Inhibition of 53BP1 favors homology-dependent DNA repair and increases CRISPR-Cas9 genome-editing efficiency. Nat. Biotechnol. *36*, 95–102.

Cao, H., Salazar-García, L., Gao, F., Wahlestedt, T., Wu, C.L., Han, X., Cai, Y., Xu, D., Wang, F., Tang, L., et al. (2019). Novel approach reveals genomic landscapes of single-strand DNA breaks with nucleotide resolution in human cells. Nat. Commun. *10*, 5799.

Cao, B., Wu, X., Zhou, J., Wu, H., Liu, L., Zhang, Q., DeMott, M.S., Gu, C., Wang, L., You, D., and Dedon, P.C. (2020). Nick-seq for single-nucleotide resolution genomic maps of DNA modifications and damage. Nucleic Acids Res. *48*, 6715–6725.

Cappelli, E., Taylor, R., Cevasco, M., Abbondandolo, A., Caldecott, K., and Frosina, G. (1997). Involvement of XRCC1 and DNA ligase III gene products in DNA base excision repair. J. Biol. Chem. *272*, 23970–23975.

Carlson-Stevermer, J., Abdeen, A.A., Kohlenberg, L., Goedland, M., Molugu, K., Lou, M., and Saha, K. (2017). Assembly of CRISPR ribonucleoproteins with biotinylated oligonucleotides via an RNA aptamer for precise gene editing. Nat. Commun. *8*, 1711.

Castanon, O., Smith, C.J., Khoshakhlagh, P., Ferreira, R., Güell, M., Said, K., Yildiz, R., Dysart, M., Wang, S., Thompson, D., et al. (2020). CRISPR-mediated biocontainment. bioRxiv. https://doi.org/10.1101/2020.02.03.922146.

Cejka, P., and Symington, L.S. (2021). DNA End Resection: Mechanism and Control. Annu. Rev. Genet. *55*, 285–307.

Chai, Y., Jiang, Y., Wang, J., Qiao, D., Zhang, Y., Xin, C., Zhou, Y., Wang, X.-C., and Chen, Q.-J. (2021). MS2 RNA aptamer enhances prime editing in rice. bioRxiv. https://doi.org/10.1101/2021.10.20.465209.

Chaikind, B., Bessen, J.L., Thompson, D.B., Hu, J.H., and Liu, D.R. (2016). A programmable Cas9-serine recombinase fusion protein that operates on DNA sequences in mammalian cells. Nucleic Acids Res. *44*, 9758–9770.

Chakrabarti, A.M., Henser-Brownhill, T., Monserrat, J., Poetsch, A.R., Luscombe, N.M., and Scaffidi, P. (2019). Target-Specific Precision of CRISPR-Mediated Genome Editing. Mol. Cell *73*, 699–713.e6.

Champer, J., Buchman, A., and Akbari, O.S. (2016). Cheating evolution: engineering gene drives to manipulate the fate of wild populations. Nat. Rev. Genet. *17*, 146–159.

Chan, K., Resnick, M.A., and Gordenin, D.A. (2013). The choice of nucleotide inserted opposite abasic sites formed within chromosomal DNA reveals the polymerase activities participating in translesion DNA synthesis. DNA Repair (Amst.) *12*, 878–889.

Chang, H.H.Y., Pannunzio, N.R., Adachi, N., and Lieber, M.R. (2017). Non-homologous DNA end joining and alternative pathways to double-strand break repair. Nat. Rev. Mol. Cell Biol. *18*, 495–506.

Chanut, P., Britton, S., Coates, J., Jackson, S.P., and Calsou, P. (2016). Coordinated nuclease activities counteract Ku at single-ended DNA double-strand breaks. Nat. Commun. *7*, 12889.

Charpentier, M., Khedher, A.H.Y., Menoret, S., Brion, A., Lamribet, K., Dardillac, E., Boix, C., Perrouault, L., Tesson, L., Geny, S., et al. (2018). CtIP fusion to Cas9 enhances transgene integration by homology-dependent repair. Nat. Commun. *9*, 1133.

Chen, S.P., and Wang, H.H. (2019). An Engineered Cas-Transposon System for Programmable and Site-Directed DNA Transpositions. CRISPR J *2*, 376–394.

Chen, X., Rinsma, M., Janssen, J.M., Liu, J., Maggio, I., and Gonçalves, M.A. (2016). Probing the impact of chromatin conformation on genome editing tools. Nucleic Acids Res. *44*, 6482–6492.

Chen, X., Janssen, J.M., Liu, J., Maggio, I., 't Jong, A.E.J., Mikkers, H.M.M., and Gonçalves, M.A.F.V. (2017a). In trans paired nicking triggers seamless genome editing without double-stranded DNA cutting. Nat. Commun. *8*, 657.

Chen, X., Liu, J., Janssen, J.M., and Gonçalves, M.A.F.V. (2017b). The Chromatin Structure Differentially Impacts High-Specificity CRISPR-Cas9 Nuclease Strategies. Mol. Ther. Nucleic Acids *8*, 558–563.

Chen, B., Zou, W., Xu, H., Liang, Y., and Huang, B. (2018a). Efficient labeling and imaging of protein-coding genes in living cells using CRISPR-Tag. Nat. Commun. *9*, 5065.

Chen, C.C., Feng, W., Lim, P.X., Kass, E.M., and Jasin, M. (2018b). Homology-Directed Repair and the Role of BRCA1, BRCA2, and Related Proteins in Genome Integrity and Cancer. Annu Rev Cancer Biol *2*, 313–336.

Chen, W., McKenna, A., Schreiber, J., Haeussler, M., Yin, Y., Agarwal, V., Noble, W.S., and Shendure, J. (2019). Massively parallel profiling and predictive modeling of the outcomes of CRISPR/Cas9-mediated double-strand break repair. Nucleic Acids Res. *47*, 7989–8003.

Chen, Y., Liu, J., Zhi, S., Zheng, Q., Ma, W., Huang, J., Liu, Y., Liu, D., Liang, P., and Songyang, Z. (2020). Repurposing type I-F CRISPR-Cas system as a transcriptional activation tool in human cells. Nat. Commun. *11*, 3136.

Chen, L., Park, J.E., Paa, P., Rajakumar, P.D., Prekop, H.T., Chew, Y.T., Manivannan, S.N., and Chew, W.L. (2021a). Programmable C:G to G:C genome editing with CRISPR-Cas9-directed base excision repair proteins. Nat. Commun. *12*, 1384.

Chen, P.J., Hussmann, J.A., Yan, J., Knipping, F., Ravisankar, P., Chen, P.-F., Chen, C., Nelson, J.W., Newby, G.A., Sahin, M., et al. (2021b). Enhanced prime editing systems by manipulating cellular determinants of editing outcomes. Cell *184*, 5635–5652.e29.

Chen, S., Lee, L., Naila, T., Fishbain, S., Wang, A., Tomkinson, A.E., Lees-Miller, S.P., and He, Y. (2021c). Structural basis of long-range to short-range synaptic transition in NHEJ. Nature *593*, 294–298.

Chen, W., Choi, J., Nathans, J.F., Agarwal, V., Martin, B., Nichols, E., Leith, A., Lee, C., and Shendure, J. (2021d). Multiplex genomic recording of enhancer and signal transduction activity in mammalian cells. bioRxiv. https://doi.org/10.1101/2021.11.05.467434.

Chen, W., Ren, Z.H., Tang, N., Chai, G., Zhang, H., Zhang, Y., Ma, J., Wu, Z., Shen, X., Huang, X., et al. (2021e). Targeted genetic screening in bacteria with a Cas12k-guided transposase. Cell Rep. *36*, 109635.

Cheng, L., Li, Y., Qi, Q., Xu, P., Feng, R., Palmer, L., Chen, J., Wu, R., Yee, T., Zhang, J., et al. (2021). Single-nucleotide-level mapping of DNA regulatory elements that control fetal hemoglobin expression. Nat. Genet. *53*, 869–880.

Cho, N.H., Cheveralls, K.C., Brunner, A.-D., Kim, K., Michaelis, A.C., Raghavan, P., Kobayashi, H., Savy, L., Li, J.Y., Canaj, H., et al. (2021). OpenCell:

proteome-scale endogenous tagging enables the cartography of human cellular organization. bioRxiv. https://doi.org/10.1101/2021.03.29.437450.

Choi, E., and Koo, T. (2021). CRISPR technologies for the treatment of Duchenne muscular dystrophy. Mol. Ther. *29*, 3179–3191.

Choi, J., Chen, W., Minkina, A., Chardon, F.M., Suiter, C.C., Regalado, S.G., Domcke, S., Hamazaki, N., Lee, C., Martin, B., et al. (2021a). A temporally resolved, multiplex molecular recorder based on sequential genome editing. bioRxiv. https://doi.org/10.1101/2021.11.05.467388.

Choi, J., Chen, W., Suiter, C.C., Lee, C., Chardon, F.M., Yang, W., Leith, A., Daza, R.M., Martin, B., and Shendure, J. (2021b). Precise genomic deletions using paired prime editing. Nat. Biotechnol. Published online October 14, 2021. https://doi.org/10.1038/s41587-021-01025-z.

Chu, V.T., Weber, T., Wefers, B., Wurst, W., Sander, S., Rajewsky, K., and Kühn, R. (2015). Increasing the efficiency of homology-directed repair for CRISPR-Cas9-induced precise gene editing in mammalian cells. Nat. Biotechnol. *33*, 543–548.

Ciccia, A., and Elledge, S.J. (2010). The DNA damage response: making it safe to play with knives. Mol. Cell *40*, 179–204.

Ciccia, A., McDonald, N., and West, S.C. (2008). Structural and functional relationships of the XPF/MUS81 family of proteins. Annu. Rev. Biochem. *77*, 259–287.

Cinesi, C., Aeschbach, L., Yang, B., and Dion, V. (2016). Contracting CAG/CTG repeats using the CRISPR-Cas9 nickase. Nat. Commun. *7*, 13272.

Constantin, N., Dzantiev, L., Kadyrov, F.A., and Modrich, P. (2005). Human mismatch repair: reconstitution of a nick-directed bidirectional reaction. J. Biol. Chem. *280*, 39752–39761.

Conticello, S.G., Langlois, M.A., Yang, Z., and Neuberger, M.S. (2007). DNA deamination in immunity: AID in the context of its APOBEC relatives. Adv. Immunol. *94*, 37–73.

Cortez, D. (2019). Replication-Coupled DNA Repair. Mol. Cell *74*, 866–876.

Cox, D.B.T., Gootenberg, J.S., Abudayyeh, O.O., Franklin, B., Kellner, M.J., Joung, J., and Zhang, F. (2017). RNA editing with CRISPR-Cas13. Science *358*, 1019–1027.

Cruz-Becerra, G., and Kadonaga, J.T. (2020). Enhancement of homology-directed repair with chromatin donor templates in cells. eLife *9*, e55780.

Csörgő, B., León, L.M., Chau-Ly, I.J., Vasquez-Rifo, A., Berry, J.D., Mahendra, C., Crawford, E.D., Lewis, J.D., and Bondy-Denomy, J. (2020). A compact Cascade-Cas3 system for targeted genome engineering. Nat. Methods *17*, 1183–1190.

Cuella-Martin, R., Hayward, S.B., Fan, X., Chen, X., Huang, J.-W., Taglialatela, A., Leuzzi, G., Zhao, J., Rabadan, R., Lu, C., et al. (2021). Functional interrogation of DNA damage response variants with base editing screens. Cell *184*, 1081–1097.e19.

Cullot, G., Boutin, J., Toutain, J., Prat, F., Pennamen, P., Rooryck, C., Teichmann, M., Rousseau, E., Lamrissi-Garcia, I., Guyonnet-Duperat, V., et al. (2019). CRISPR-Cas9 genome editing induces megabase-scale chromosomal truncations. Nat. Commun. *10*, 1136.

Daer, R.M., Cutts, J.P., Brafman, D.A., and Haynes, K.A. (2017). The Impact of Chromatin Dynamics on Cas9-Mediated Genome Editing in Human Cells. ACS Synth. Biol. *6*, 428–438.

Dahiya, R., Hu, Q., and Ly, P. (2021). Mechanistic origins of diverse genome rearrangements in cancer. Semin. Cell Dev. Biol. Published online April 3, 2021. https://doi.org/10.1016/j.semcdb.2021.03.003.

Dalvai, M., Loehr, J., Jacquet, K., Huard, C.C., Roques, C., Herst, P., Côté, J., and Doyon, Y. (2015). A Scalable Genome-Editing-Based Approach for Mapping Multiprotein Complexes in Human Cells. Cell Rep. *13*, 621–633.

Davis, A.J., and Chen, D.J. (2013). DNA double strand break repair via non-homologous end-joining. Transl. Cancer Res. *2*, 130–143.

Davis, L., and Maizels, N. (2014). Homology-directed repair of DNA nicks via pathways distinct from canonical double-strand break repair. Proc. Natl. Acad. Sci. USA *111*, E924–E932.

Davis, L., and Maizels, N. (2016). Two Distinct Pathways Support Gene Correction by Single-Stranded Donors at DNA Nicks. Cell Rep. *17*, 1872–1881.

de Moraes, M.H., Hsu, F., Huang, D., Bosch, D.E., Zeng, J., Radey, M.C., Simon, N., Ledvina, H.E., Frick, J.P., Wiggins, P.A., et al. (2021). An interbacterial DNA deaminase toxin directly mutagenizes surviving target populations. eLife *10*, e62967.

Demin, A.A., Hirota, K., Tsuda, M., Adamowicz, M., Hailstone, R., Brazina, J., Gittens, W., Kalasova, I., Shao, Z., Zha, S., et al. (2021). XRCC1 prevents toxic PARP1 trapping during DNA base excision repair. Mol. Cell *81*, 3018–3030.e5.

Deshpande, R.A., Myler, L.R., Soniat, M.M., Makharashvili, N., Lee, L., Lees-Miller, S.P., Finkelstein, I.J., and Paull, T.T. (2020). DNA-dependent protein kinase promotes DNA end processing by MRN and CtIP. Sci. Adv. *6*, y0922.

Després, P.C., Dubé, A.K., Seki, M., Yachie, N., and Landry, C.R. (2020). Perturbing proteomes at single residue resolution using base editing. Nat. Commun. *11*, 1871.

Doron, S., Melamed, S., Ofir, G., Leavitt, A., Lopatina, A., Keren, M., Amitai, G., and Sorek, R. (2018). Systematic discovery of antiphage defense systems in the microbial pangenome. Science *359*, eaar4120.

Doudna, J.A. (2020). The promise and challenge of therapeutic genome editing. Nature *578*, 229–236.

Durrant, M.G., Fanton, A., Tycko, J., Hinks, M., Chandrasekaran, S., Perry, N.T., Schaepe, J., Du, P.P., Bintu, L., Bassik, M.C., et al. (2021). Large-scale discovery of recombinases for integrating DNA into the human genome. bioRxiv. https://doi.org/10.1101/2021.11.05.467528.

Egli, D., Zuccaro, M.V., Kosicki, M., Church, G.M., Bradley, A., and Jasin, M. (2018). Inter-homologue repair in fertilized human eggs? Nature *560*, E5–E7.

Elacqua, J.J., Ranu, N., Dilorio, S.E., and Blainey, P.C. (2021). DENT-seq for genome-wide strand-specific identification of DNA single-strand break sites with single-nucleotide resolution. Genome Res. *31*, 75–87.

Ellis, G.I., Sheppard, N.C., and Riley, J.L. (2021). Genetic engineering of T cells for immunotherapy. Nat. Rev. Genet. *22*, 427–447.

Enache, O.M., Rendo, V., Abdusamad, M., Lam, D., Davison, D., Pal, S., Currimjee, N., Hess, J., Pantel, S., Nag, A., et al. (2020). Cas9 activates the p53 pathway and selects for p53-inactivating mutations. Nat. Genet. *52*, 662–668.

Erwood, S., Bily, T.M.I., Lequyer, J., Yan, J., Gulati, N., Brewer, R.A., Zhou, L., Pelletier, L., Ivakine, E.A., and Cohn, R.D. (2021). Saturation variant interpretation using CRISPR prime editing. bioRxiv. https://doi.org/10.1101/2021.05.11.443710.

Eyquem, J., Mansilla-Soto, J., Giavridis, T., van der Stegen, S.J., Hamieh, M., Cunanan, K.M., Odak, A., Gönen, M., and Sadelain, M. (2017). Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature *543*, 113–117.

Farzadfard, F., Gharaei, N., Citorik, R.J., and Lu, T.K. (2021). Efficient retroelement-mediated DNA writing in bacteria. Cell Syst. *12*, 860–872.e5.

Faure, G., Shmakov, S.A., Yan, W.X., Cheng, D.R., Scott, D.A., Peters, J.E., Makarova, K.S., and Koonin, E.V. (2019). CRISPR-Cas in mobile genetic elements: counter-defence and beyond. Nat. Rev. Microbiol. *17*, 513–525.

Ferrari, S., Jacob, A., Beretta, S., Unali, G., Albano, L., Vavassori, V., Cittaro, D., Lazarevic, D., Brombin, C., Cugnata, F., et al. (2020). Efficient gene editing of human long-term hematopoietic stem cells validated by clonal tracking. Nat. Biotechnol. *38*, 1298–1308.

Ferrari, G., Thrasher, A.J., and Aiuti, A. (2021). Gene therapy using haematopoietic stem and progenitor cells. Nat. Rev. Genet. *22*, 216–234.

Ferreira da Silva, J., Meyenberg, M., and Loizou, J.I. (2021a). Tissue specificity of DNA repair: the CRISPR compass. Trends Genet. *37*, 958–962.

Ferreira da Silva, J., Oliveira, G., Arasa-Verge, E., Moretton, A., Thimelthaler, G., Jiricny, J., and Loizou, J.I. (2021b). Prime Editing Efficiency and Fidelity are Enhanced in the Absence of Mismatch Repair. bioRxiv. https://doi.org/10.1101/2021.09.30.462548.

Filsinger, G.T., Wannier, T.M., Pedersen, F.B., Lutz, I.D., Zhang, J., Stork, D.A., Debnath, A., Gozzi, K., Kuchwara, H., Volf, V., et al. (2021). Characterizing the

portability of phage-encoded homologous recombination proteins. Nat. Chem. Biol. *17*, 394–402.

Findlay, G.M., Boyle, E.A., Hause, R.J., Klein, J.C., and Shendure, J. (2014). Saturation editing of genomic regions by multiplex homology-directed repair. Nature *513*, 120–123.

Findlay, G.M., Daza, R.M., Martin, B., Zhang, M.D., Leith, A.P., Gasperini, M., Janizek, J.D., Huang, X., Starita, L.M., and Shendure, J. (2018). Accurate classification of BRCA1 variants with saturation genome editing. Nature *562*, 217–222.

Fonfara, I., Richter, H., Bratovič, M., Le Rhun, A., and Charpentier, E. (2016). The CRISPR-associated DNA-cleaving enzyme Cpf1 also processes precursor CRISPR RNA. Nature *532*, 517–521.

Fradet-Turcotte, A., Canny, M.D., Escribano-Díaz, C., Orthwein, A., Leung, C.C., Huang, H., Landry, M.C., Kitevski-LeBlanc, J., Noordermeer, S.M., Sicheri, F., and Durocher, D. (2013). 53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark. Nature *499*, 50–54.

Frangoul, H., Altshuler, D., Cappellini, M.D., Chen, Y.S., Domm, J., Eustace, B.K., Foell, J., de la Fuente, J., Grupp, S., Handgretinger, R., et al. (2021). CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. N. Engl. J. Med. *384*, 252–260.

Frit, P., Ropars, V., Modesti, M., Charbonnier, J.B., and Calsou, P. (2019). Plugged into the Ku-DNA hub: The NHEJ network. Prog. Biophys. Mol. Biol. *147*, 62–76.

Frock, R.L., Hu, J., Meyers, R.M., Ho, Y.J., Kii, E., and Alt, F.W. (2015). Genome-wide detection of DNA double-stranded breaks induced by engineered nucleases. Nat. Biotechnol. *33*, 179–186.

Fu, Y., Tigano, M., and Sfeir, A. (2020). Safeguarding mitochondrial genomes in higher eukaryotes. Nat. Struct. Mol. Biol. *27*, 687–695.

Fu, J., Li, Q., Liu, X., Tu, T., Lv, X., Yin, X., Lv, J., Song, Z., Qu, J., Zhang, J., et al. (2021). Human cell based directed evolution of adenine base editors with improved efficiency. Nat. Commun. *12*, 5897.

Gallagher, D.N., and Haber, J.E. (2018). Repair of a Site-Specific DNA Cleavage: Old-School Lessons for Cas9-Mediated Gene Editing. ACS Chem. Biol. *13*, 397–405.

Gallagher, D.N., Pham, N., Tsai, A.M., Janto, A.N., Choi, J., Ira, G., and Haber, J.E. (2020). A Rad51-independent pathway promotes single-strand template repair in gene editing. PLoS Genet. *16*, e1008689.

Gammage, P.A., Moraes, C.T., and Minczuk, M. (2018). Mitochondrial Genome Engineering: The Revolution May Not Be CRISPR-Ized. Trends Genet. *34*, 101–110.

Gao, L., Altae-Tran, H., Böhning, F., Makarova, K.S., Segel, M., Schmid-Burgk, J.L., Koob, J., Wolf, Y.I., Koonin, E.V., and Zhang, F. (2020). Diverse enzymatic activities mediate antiviral immunity in prokaryotes. Science *369*, 1077–1084.

Gao, R., Fu, Z.-C., Li, X., Wang, Y., Wei, J., Li, G., Wang, L., Wu, J., Xue, W., Huang, X., et al. (2021a). No observable guide-RNA-independent off-target mutation induced by prime. bioRxiv. https://doi.org/10.1101/2021.04.09.439109.

Gao, Z., Haldrup, J., Ravendran, S., Mikkelsen, N.S., Mikkelsen, J.G., and Bak, R.O. (2021b). A truncated reverse transcriptase enhances prime editing by split AAV vectors. bioRxiv. https://doi.org/10.1101/2021.11.05.467423.

Gapinske, M., Luu, A., Winter, J., Woods, W.S., Kostan, K.A., Shiva, N., Song, J.S., and Perez-Pinera, P. (2018). CRISPR-SKIP: programmable gene splicing with single base. Genome Biol. *19*, 107.

Garneau, J.E., Dupuis, M.E., Villion, M., Romero, D.A., Barrangou, R., Boyaval, P., Fremaux, C., Horvath, P., Magadán, A.H., and Moineau, S. (2010). The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature *468*, 67–71.

Gasiunas, G., Barrangou, R., Horvath, P., and Siksnys, V. (2012). Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc. Natl. Acad. Sci. USA *109*, E2579–E2586.

Gaudelli, N.M., Komor, A.C., Rees, H.A., Packer, M.S., Badran, A.H., Bryson, D.I., and Liu, D.R. (2017). Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. Nature *551*, 464–471.

Gaudelli, N.M., Lam, D.K., Rees, H.A., Solá-Esteves, N.M., Barrera, L.A., Born, D.A., Edwards, A., Gehrke, J.M., Lee, S.J., Liquori, A.J., et al. (2020). Directed evolution of adenine base editors with increased activity and therapeutic application. Nat. Biotechnol. *38*, 892–900.

Gehrke, J.M., Cervantes, O., Clement, M.K., Wu, Y., Zeng, J., Bauer, D.E., Pinello, L., and Joung, J.K. (2018). An APOBEC3A-Cas9 base editor with minimized bystander and off-target activities. Nat. Biotechnol. *36*, 977–982.

Gersbach, C.A., Gaj, T., Gordley, R.M., Mercer, A.C., and Barbas, C.F., 3rd (2011). Targeted plasmid integration into the human genome by an engineered zinc-finger recombinase. Nucleic Acids Res. *39*, 7868–7878.

Gillmore, J.D., Gane, E., Taubel, J., Kao, J., Fontana, M., Maitland, M.L., Seitzer, J., O'Connell, D., Walsh, K.R., Wood, K., et al. (2021). CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis. N. Engl. J. Med. *385*, 493–502.

Goellner, E.M., Putnam, C.D., and Kolodner, R.D. (2015). Exonuclease 1-dependent and independent mismatch repair. DNA Repair (Amst.) *32*, 24–32.

Gonçalves, M.A., van Nierop, G.P., Holkers, M., and de Vries, A.A. (2012). Concerted nicking of donor and chromosomal acceptor DNA promotes homology-directed gene targeting in human cells. Nucleic Acids Res. *40*, 3443–3455.

Gordley, R.M., Gersbach, C.A., and Barbas, C.F., 3rd (2009). Synthesis of programmable integrases. Proc. Natl. Acad. Sci. USA *106*, 5053–5058.

Graham, T.G., Walter, J.C., and Loparo, J.J. (2016). Two-Stage Synapsis of DNA Ends during Non-homologous End Joining. Mol. Cell *61*, 850–858.

Greco, G.E., Matsumoto, Y., Brooks, R.C., Lu, Z., Lieber, M.R., and Tomkinson, A.E. (2016). SCR7 is neither a selective nor a potent inhibitor of human DNA ligase IV. DNA Repair (Amst.) *43*, 18–23.

Grünewald, J., Zhou, R., Lareau, C.A., Garcia, S.P., Iyer, S., Miller, B.R., Langner, L.M., Hsu, J.Y., Aryee, M.J., and Joung, J.K. (2020). A dual-deaminase CRISPR base editor enables concurrent adenine and cytosine editing. Nat. Biotechnol. *38*, 861–864.

Gu, B., Posfai, E., and Rossant, J. (2018). Efficient generation of targeted large insertions by microinjection into two-cell-stage mouse embryos. Nat. Biotechnol. *36*, 632–637.

Gu, S., Bodai, Z., Cowan, Q.T., and Komor, A.C. (2021). Base editors: Expanding the types of DNA damage products harnessed for genome editing. Gene Genome Ed *1*, 100005.

Guo, Q., Mintier, G., Ma-Edmonds, M., Storton, D., Wang, X., Xiao, X., Kienzle, B., Zhao, D., and Feder, J.N. (2018). 'Cold shock' increases the frequency of homology directed repair gene editing in induced pluripotent stem cells. Sci. Rep. *8*, 2080.

Gutierrez-Triana, J.A., Tavhelidse, T., Thumberger, T., Thomas, I., Wittbrodt, B., Kellner, T., Anlas, K., Tsingos, E., and Wittbrodt, J. (2018). Efficient single-copy HDR by 5′ modified long dsDNA donors. eLife *7*, e39468.

Gutschner, T., Haemmerle, M., Genovese, G., Draetta, G.F., and Chin, L. (2016). Post-translational Regulation of Cas9 during G1 Enhances Homology-Directed Repair. Cell Rep. *14*, 1555–1566.

Haapaniemi, E., Botla, S., Persson, J., Schmierer, B., and Taipale, J. (2018). CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response. Nat. Med. *24*, 927–930.

Habib, O., Habib, G., Hwang, G.-H., and Bae, S. (2021). Comprehensive analysis of prime editing outcomes in human embryonic stem cells. bioRxiv. https://doi.org/10.1101/2021.04.12.439533.

Hackley, C.R. (2021). A Novel Set of Cas9 Fusion Proteins to Stimulate Homologous Recombination: Cas9-HRs. CRISPR J *4*, 253–263.

Halperin, S.O., Tou, C.J., Wong, E.B., Modavi, C., Schaffer, D.V., and Dueber, J.E. (2018). CRISPR-guided DNA polymerases enable diversification of all nucleotides in a tunable window. Nature *560*, 248–252.

Halpin-Healy, T.S., Klompe, S.E., Sternberg, S.H., and Fernández, I.S. (2020). Structural basis of DNA targeting by a transposon-encoded CRISPR-Cas system. Nature *577*, 271–274.

Hampton, H.G., Watson, B.N.J., and Fineran, P.C. (2020). The arms race between bacteria and their phage foes. Nature *577*, 327–336.

Hanna, R.E., Hegde, M., Fagre, C.R., DeWeirdt, P.C., Sangree, A.K., Szegletes, Z., Griffith, A., Feeley, M.N., Sanson, K.R., Baidi, Y., et al. (2021). Massively parallel assessment of human variants with base editor screens. Cell *184*, 1064–1080.e20.

Harel, I., Valenzano, D.R., and Brunet, A. (2016). Efficient genome engineering approaches for the short-lived African turquoise killifish. Nat. Protoc. *11*, 2010–2028.

Harrington, L.B., Burstein, D., Chen, J.S., Paez-Espino, D., Ma, E., Witte, I.P., Cofsky, J.C., Kyrpides, N.C., Banfield, J.F., and Doudna, J.A. (2018). Programmed DNA destruction by miniature CRISPR-Cas14 enzymes. Science *362*, 839–842.

Hastings, P.J., Ira, G., and Lupski, J.R. (2009). A microhomology-mediated break-induced replication model for the origin of human copy number variation. PLoS Genet. *5*, e1000327.

Hayward, S.B., and Ciccia, A. (2021). Towards a CRISPeR understanding of homologous recombination with high-throughput functional genomics. Curr. Opin. Genet. Dev. *71*, 171–181.

Hess, G.T., Frésard, L., Han, K., Lee, C.H., Li, A., Cimprich, K.A., Montgomery, S.B., and Bassik, M.C. (2016). Directed evolution using dCas9-targeted somatic hypermutation in mammalian cells. Nat. Methods *13*, 1036–1042.

Hickman, A.B., and Dyda, F. (2016). DNA Transposition at Work. Chem. Rev. *116*, 12758–12784.

High, K.A., and Roncarolo, M.G. (2019). Gene Therapy. N. Engl. J. Med. *381*, 455–464.

Hirotsune, S., Kiyonari, H., Jin, M., Kumamoto, K., Yoshida, K., Shinohara, M., Watanabe, H., Wynshaw-Boris, A., and Matsuzaki, F. (2020). Enhanced homologous recombination by the modulation of targeting vector ends. Sci. Rep. *10*, 2518.

Horlbeck, M.A., Witkowsky, L.B., Guglielmi, B., Replogle, J.M., Gilbert, L.A., Villalta, J.E., Torigoe, S.E., Tjian, R., and Weissman, J.S. (2016). Nucleosomes impede Cas9 access to DNA in vivo and in vitro. eLife *5*, e12677.

Hsieh, P., and Zhang, Y. (2017). The Devil is in the details for DNA mismatch repair. Proc. Natl. Acad. Sci. USA *114*, 3552–3554.

Hu, J.H., Miller, S.M., Geurts, M.H., Tang, W., Chen, L., Sun, N., Zeina, C.M., Gao, X., Rees, H.A., Lin, Z., et al. (2018a). Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. Nature *556*, 57–63.

Hu, Z., Shi, Z., Guo, X., Jiang, B., Wang, G., Luo, D., Chen, Y., and Zhu, Y.S. (2018b). Ligase IV inhibitor SCR7 enhances gene editing directed by CRISPR-Cas9 and ssODN in human cancer cells. Cell Biosci. *8*, 12.

Huang, C., Li, G., Wu, J., Liang, J., and Wang, X. (2021a). Identification of pathogenic variants in cancer genes using base editing screens with editing efficiency correction. Genome Biol. *22*, 80.

Huang, T.P., Newby, G.A., and Liu, D.R. (2021b). Precision genome editing using cytosine and adenine base editors in mammalian cells. Nat. Protoc. *16*, 1089–1128.

Hug, L.A., Baker, B.J., Anantharaman, K., Brown, C.T., Probst, A.J., Castelle, C.J., Butterfield, C.N., Hernsdorf, A.W., Amano, Y., Ise, K., et al. (2016). A new view of the tree of life. Nat. Microbiol. *1*, 16048.

Hussmann, J.A., Ling, J., Ravisankar, P., Yan, J., Cirincione, A., Xu, A., Simpson, D., Yang, D., Bothmer, A., Cotta-Ramusino, C., et al. (2021). Mapping the genetic landscape of DNA double-strand break repair. Cell *184*, 5653–5669.e25.

Hustedt, N., and Durocher, D. (2016). The control of DNA repair by the cell cycle. Nat. Cell Biol. *19*, 1–9.

Hyodo, T., Rahman, M.L., Karnan, S., Ito, T., Toyoda, A., Ota, A., Wahiduzzaman, M., Tsuzuki, S., Okada, Y., Hosokawa, Y., and Konishi, H. (2020). Tandem Paired Nicking Promotes Precise Genome Editing with Scarce Interference by p53. Cell Rep. *30*, 1195–1207.e7.

Ihry, R.J., Worringer, K.A., Salick, M.R., Frias, E., Ho, D., Theriault, K., Kommineni, S., Chen, J., Sondey, M., Ye, C., et al. (2018). p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. Nat. Med. *24*, 939–946.

Ioannidi, E.I., Yarnall, M.T.N., Schmitt-Ulms, C., Krajeski, R.N., Lim, J., Villiger, L., Zhou, W., Jiang, K., Roberts, N., Zhang, L., et al. (2021). Drag-and-drop genome insertion without DNA cleavage with CRISPR-directed integrases. bioRxiv. https://doi.org/10.1101/2021.11.01.466786.

Iyer, S., Suresh, S., Guo, D., Daman, K., Chen, J.C.J., Liu, P., Zieger, M., Luk, K., Roscoe, B.P., Mueller, C., et al. (2019). Precise therapeutic gene correction by a simple nuclease-induced double-stranded break. Nature *568*, 561–565.

Jang, H., Jo, D.H., Cho, C.S., Shin, J.H., Seo, J.H., Yu, G., Gopalappa, R., Kim, D., Cho, S.-R., Kim, J.H., and Kim, H.H. (2021). Application of prime editing to the correction of mutations and phenotypes in adult mice with liver and eye diseases. Nat. Biomed. Eng. Published online on August 26, 2021. https://doi.org/10.1038/s41551-021-00788-9.

Jansen, J.G., Langerak, P., Tsaalbi-Shtylik, A., van den Berk, P., Jacobs, H., and de Wind, N. (2006). Strand-biased defect in C/G transversions in hypermutating immunoglobulin genes in Rev1-deficient mice. J. Exp. Med. *203*, 319–323.

Jasin, M., and Haber, J.E. (2016). The democratization of gene editing: Insights from site-specific cleavage and double-strand break repair. DNA Repair (Amst.) *44*, 6–16.

Jayavaradhan, R., Pillis, D.M., Goodman, M., Zhang, F., Zhang, Y., Andreassen, P.R., and Malik, P. (2019). CRISPR-Cas9 fusion to dominant-negative 53BP1 enhances HDR and inhibits NHEJ specifically at Cas9 target sites. Nat. Commun. *10*, 2866.

Jensen, K.T., Fløe, L., Petersen, T.S., Huang, J., Xu, F., Bolund, L., Luo, Y., and Lin, L. (2017). Chromatin accessibility and guide sequence secondary structure affect CRISPR-Cas9 gene editing efficiency. FEBS Lett. *591*, 1892–1901.

Jeong, Y.K., Lee, S., Hwang, G.H., Hong, S.A., Park, S.E., Kim, J.S., Woo, J.S., and Bae, S. (2021). Adenine base editor engineering reduces editing of bystander cytosines. Nat. Biotechnol. *39*, 1426–1433.

Jiang, T., Zhang, X.-O., Weng, Z., and Xue, W. (2021). Deletion and replacement of long genomic sequences using prime editing. Nat. Biotechnol. Published online October 14, 2021. https://doi.org/10.1038/s41587-021-01026-y.

Jin, S., Lin, Q., Luo, Y., Zhu, Z., Liu, G., Li, Y., Chen, K., Qiu, J.L., and Gao, C. (2021). Genome-wide specificity of prime editors in plants. Nat. Biotechnol. *39*, 1292–1299.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., and Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science *337*, 816–821.

Jiricny, J. (2013). Postreplicative mismatch repair. Cold Spring Harb. Perspect. Biol. *5*, a012633.

Jun, S., Lim, H., Chun, H., Lee, J.H., and Bang, D. (2020). Single-cell analysis of a mutant library generated using CRISPR-guided deaminase in human melanoma cells. Commun Biol *3*, 154.

Kan, Y., Ruis, B., Lin, S., and Hendrickson, E.A. (2014). The mechanism of gene targeting in human somatic cells. PLoS Genet. *10*, e1004251.

Kan, Y., Ruis, B., Takasugi, T., and Hendrickson, E.A. (2017). Mechanisms of precise genome editing using oligonucleotide donors. Genome Res. *27*, 1099–1111.

Karvelis, T., Bigelyte, G., Young, J.K., Hou, Z., Zedaveinyte, R., Budre, K., Paulraj, S., Djukanovic, V., Gasior, S., Silanskas, A., et al. (2020). PAM recognition by miniature CRISPR-Cas12f nucleases triggers programmable double-stranded DNA target cleavage. Nucleic Acids Res. *48*, 5016–5023.

Karvelis, T., Druteika, G., Bigelyte, G., Budre, K., Zedaveinyte, R., Silanskas, A., Kazlauskas, D., Venclovas, C., and Siksnys, V. (2021). Transposon-associated TnpB is a programmable RNA-guided DNA endonuclease. Nature *599*, 692–696.

Keskin, H., Shen, Y., Huang, F., Patel, M., Yang, T., Ashley, K., Mazin, A.V., and Storici, F. (2014). Transcript-RNA-templated DNA recombination and repair. Nature *515*, 436–439.

Kim, H., and Kim, J.-S. (2014). A guide to genome engineering with programmable nucleases. Nat. Rev. Genet. *15*, 321–334.

Kim, N., Mudrak, S.V., and Jinks-Robertson, S. (2011). The dCMP transferase activity of yeast Rev1 is biologically relevant during the bypass of endogenously generated AP sites. DNA Repair (Amst.) *10*, 1262–1271.

Kim, K., Ryu, S.M., Kim, S.T., Baek, G., Kim, D., Lim, K., Chung, E., Kim, S., and Kim, J.S. (2017a). Highly efficient RNA-guided base editing in mouse embryos. Nat. Biotechnol. *35*, 435–437.

Kim, Y.B., Komor, A.C., Levy, J.M., Packer, M.S., Zhao, K.T., and Liu, D.R. (2017b). Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions. Nat. Biotechnol. *35*, 371–376.

Kim, H.S., Jeong, Y.K., Hur, J.K., Kim, J.S., and Bae, S. (2019). Adenine base editors catalyze cytosine conversions in human cells. Nat. Biotechnol. *37*, 1145–1148.

Kim, D.Y., Lee, J.M., Moon, S.B., Chin, H.J., Park, S., Lim, Y., Kim, D., Koo, T., Ko, J.H., and Kim, Y.S. (2021a). Efficient CRISPR editing with a hypercompact Cas12f1 and engineered guide RNAs delivered by adeno-associated virus. Nat. Biotechnol. Published online September 2, 2021. https://doi.org/10.1038/s41587-021-01009-z.

Kim, H.K., Yu, G., Park, J., Min, S., Lee, S., Yoon, S., and Kim, H.H. (2021b). Predicting the efficiency of prime editing guide RNAs in human cells. Nat. Biotechnol. *39*, 198–206.

Kleinstiver, B.P., Sousa, A.A., Walton, R.T., Tak, Y.E., Hsu, J.Y., Clement, K., Welch, M.M., Horng, J.E., Malagon-Lopez, J., Scarfò, I., et al. (2019). Engineered CRISPR-Cas12a variants with increased activities and improved targeting ranges for gene, epigenetic and base editing. Nat. Biotechnol. *37*, 276–282.

Klompe, S.E., Vo, P.L.H., Halpin-Healy, T.S., and Sternberg, S.H. (2019). Transposon-encoded CRISPR-Cas systems direct RNA-guided DNA integration. Nature *571*, 219–225.

Kluesner, M.G., Lahr, W.S., Lonetree, C.L., Smeester, B.A., Qiu, X., Slipek, N.J., Claudio Vázquez, P.N., Pitzen, S.P., Pomeroy, E.J., Vignes, M.J., et al. (2021). CRISPR-Cas9 cytidine and adenosine base editing of splice-sites mediates highly-efficient disruption of proteins in primary and immortalized cells. Nat. Commun. *12*, 2437.

Knott, G.J., and Doudna, J.A. (2018). CRISPR-Cas guides the future of genetic engineering. Science *361*, 866–869.

Koblan, L.W., Doman, J.L., Wilson, C., Levy, J.M., Tay, T., Newby, G.A., Maianti, J.P., Raguram, A., and Liu, D.R. (2018). Improving cytidine and adenine base editors by expression optimization and ancestral reconstruction. Nat. Biotechnol. *36*, 843–846.

Koblan, L.W., Arbab, M., Shen, M.W., Hussmann, J.A., Anzalone, A.V., Doman, J.L., Newby, G.A., Yang, D., Mok, B., Replogle, J.M., et al. (2021a). Efficient C•G-to-G•C base editors developed using CRISPRi screens, target-library analysis, and machine learning. Nat. Biotechnol. *39*, 1414–1425.

Koblan, L.W., Erdos, M.R., Wilson, C., Cabral, W.A., Levy, J.M., Xiong, Z.M., Tavarez, U.L., Davison, L.M., Gete, Y.G., Mao, X., et al. (2021b). In vivo base editing rescues Hutchinson-Gilford progeria syndrome in mice. Nature *589*, 608–614.

Koeppel, J., Peets, E.M., Weller, J., Pallaseni, A., Liberante, F., and Parts, L. (2021). Predicting efficiency of writing short sequences into the genome using prime editing. bioRxiv. https://doi.org/10.1101/2021.11.10.468024.

Komor, A.C., Kim, Y.B., Packer, M.S., Zuris, J.A., and Liu, D.R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature *533*, 420–424.

Komor, A.C., Zhao, K.T., Packer, M.S., Gaudelli, N.M., Waterbury, A.L., Koblan, L.W., Kim, Y.B., Badran, A.H., and Liu, D.R. (2017). Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity. Sci. Adv. *3*, o4774.

Koonin, E.V., Makarova, K.S., and Zhang, F. (2017). Diversity, classification and evolution of CRISPR-Cas systems. Curr. Opin. Microbiol. *37*, 67–78.

Kosicki, M., Tomberg, K., and Bradley, A. (2018). Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. Nat. Biotechnol. *36*, 765–771.

Kosicki, M., Allen, F., and Bradley, A. (2020). Cas9-induced large deletions and small indels are controlled in a convergent fashion. bioRxiv. https://doi.org/10.1101/2020.08.05.216739.

Kramara, J., Osia, B., and Malkova, A. (2018). Break-Induced Replication: The Where, The Why, and The How. Trends Genet. *34*, 518–531.

Krokan, H.E., and Bjørås, M. (2013). Base excision repair. Cold Spring Harb. Perspect. Biol. *5*, a012583.

Kulkarni, J.A., Witzigmann, D., Thomson, S.B., Chen, S., Leavitt, B.R., Cullis, P.R., and van der Meel, R. (2021). The current landscape of nucleic acid therapeutics. Nat. Nanotechnol. *16*, 630–643.

Kunkel, T.A., and Erie, D.A. (2015). Eukaryotic Mismatch Repair in Relation to DNA Replication. Annu. Rev. Genet. *49*, 291–313.

Kurt, I.C., Zhou, R., Iyer, S., Garcia, S.P., Miller, B.R., Langner, L.M., Grünewald, J., and Joung, J.K. (2021). CRISPR C-to-G base editors for inducing targeted DNA transversions in human cells. Nat. Biotechnol. *39*, 41–46.

Kuscu, C., Parlak, M., Tufan, T., Yang, J., Szlachta, K., Wei, X., Mammadov, R., and Adli, M. (2017). CRISPR-STOP: gene silencing through base-editing-induced nonsense mutations. Nat. Methods *14*, 710–712.

Kuzminov, A. (2001). Single-strand interruptions in replicating chromosomes cause double-strand breaks. Proc. Natl. Acad. Sci. USA *98*, 8241–8246.

Kweon, J., Jang, A.-H., Shin, H.R., See, J.-E., Lee, W., Lee, J.W., Chang, S., Kim, K., and Kim, Y. (2020). A CRISPR-based base-editing screen for the functional assessment of BRCA1 variants. Oncogene *39*, 30–35.

Kweon, J., Yoon, J.K., Jang, A.H., Shin, H.R., See, J.E., Jang, G., Kim, J.I., and Kim, Y. (2021). Engineered prime editors with PAM flexibility. Mol. Ther. *29*, 2001–2007.

Lapinaite, A., Knott, G.J., Palumbo, C.M., Lin-Shiao, E., Richter, M.F., Zhao, K.T., Beal, P.A., Liu, D.R., and Doudna, J.A. (2020). DNA capture by a CRISPR-Cas9-guided adenine base. Science *369*, 566–571.

Lee, K., Mackley, V.A., Rao, A., Chong, A.T., Dewitt, M.A., Corn, J.E., and Murthy, N. (2017). Synthetically modified guide RNA and donor DNA are a versatile platform for CRISPR-Cas9 engineering. eLife *6*, e25312.

Lee, H., Lee, S., Baek, G., Kim, A., Kang, B.C., Seo, H., and Kim, J.S. (2021). Mitochondrial DNA editing in mice with DddA-TALE fusion deaminases. Nat. Commun. *12*, 1190.

Leenay, R.T., Aghazadeh, A., Hiatt, J., Tse, D., Roth, T.L., Apathy, R., Shifrut, E., Hultquist, J.F., Krogan, N., Wu, Z., et al. (2019). Large dataset enables prediction of repair after CRISPR-Cas9 editing in primary T cells. Nat. Biotechnol. *37*, 1034–1037.

Lei, L., Chen, H., Xue, W., Yang, B., Hu, B., Wei, J., Wang, L., Cui, Y., Li, W., Wang, J., et al. (2018). APOBEC3 induces mutations during repair of CRISPR-Cas9-generated DNA breaks. Nat. Struct. Mol. Biol. *25*, 45–52.

Leibowitz, M.L., Papathanasiou, S., Doerfler, P.A., Blaine, L.J., Sun, L., Yao, Y., Zhang, C.-Z., Weiss, M.J., and Pellman, D. (2021). Chromothripsis as an on-target consequence of CRISPR-Cas9 genome editing. Nat. Genet. *53*, 895–905.

Leonetti, M.D., Sekine, S., Kamiyama, D., Weissman, J.S., and Huang, B. (2016). A scalable strategy for high-throughput GFP tagging of endogenous human proteins. Proc. Natl. Acad. Sci. USA *113*, E3501–E3508.

Levesque, S., Mayorga, D., Fiset, J.-P., Goupil, C., Duringer, A., Loiselle, A., Bouchard, E., Agudelo, D., and Doyon, Y. (2021). Marker-free coselection for successive rounds of prime editing in human cells. bioRxiv. https://doi.org/10.1101/2021.11.02.464583.

Levy, J.M., Yeh, W.H., Pendse, N., Davis, J.R., Hennessey, E., Butcher, R., Koblan, L.W., Comander, J., Liu, Q., and Liu, D.R. (2020). Cytosine and adenine base editing of the brain, liver, retina, heart and skeletal muscle of mice via adeno-associated viruses. Nat. Biomed. Eng. *4*, 97–110.

Li, P.P., and Margolis, R.L. (2021). Use of single guided Cas9 nickase to facilitate precise and efficient genome editing in human iPSCs. Sci. Rep. *11*, 9865.

Li, G., Zhang, X., Zhong, C., Mo, J., Quan, R., Yang, J., Liu, D., Li, Z., Yang, H., and Wu, Z. (2017). Small molecules enhance CRISPR/Cas9-mediated homology-directed genome editing in primary cells. Sci. Rep. *7*, 8943.

Li, Q., Li, Y., Yang, S., Huang, S., Yan, M., Ding, Y., Tang, W., Lou, X., Yin, Q., Sun, Z., et al. (2018a). CRISPR-Cas9-mediated base-editing screening in mice identifies DND1 amino acids that are critical for primordial germ cell development. Nat. Cell Biol. *20*, 1315–1325.

Li, X., Wang, Y., Liu, Y., Yang, B., Wang, X., Wei, J., Lu, Z., Zhang, Y., Wu, J., Huang, X., et al. (2018b). Base editing with a Cpf1-cytidine deaminase fusion. Nat. Biotechnol. *36*, 324–327.

Li, X.L., Li, G.H., Fu, J., Fu, Y.W., Zhang, L., Chen, W., Arakaki, C., Zhang, J.P., Wen, W., Zhao, M., et al. (2018c). Highly efficient genome editing via CRISPR-Cas9 in human pluripotent stem cells is achieved by transient BCL-XL overexpression. Nucleic Acids Res. *46*, 10195–10215.

Li, H., Beckman, K.A., Pessino, V., Huang, B., Weissman, J.S., and Leonetti, M.D. (2019). Design and specificity of long ssDNA donors for CRISPR-based knock-in. bioRxiv,. https://doi.org/10.1101/178905.

Li, C., Zhang, R., Meng, X., Chen, S., Zong, Y., Lu, C., Qiu, J.L., Chen, Y.H., Li, J., and Gao, C. (2020a). Targeted, random mutagenesis of plant genes with dual cytosine and adenine base editors. Nat. Biotechnol. *38*, 875–882.

Li, G., Zhang, X., Wang, H., Liu, D., Li, Z., Wu, Z., and Yang, H. (2020b). Increasing CRISPR/Cas9-mediated homology-directed DNA repair by histone deacetylase inhibitors. Int. J. Biochem. Cell Biol. *125*, 105790.

Liang, X., Potter, J., Kumar, S., Ravinder, N., and Chesnut, J.D. (2017). Enhanced CRISPR/Cas9-mediated precise genome editing by improved design and delivery of gRNA, Cas9 nuclease, and donor DNA. J. Biotechnol. *241*, 136–146.

Liao, C., and Beisel, C.L. (2021). The tracrRNA in CRISPR Biology and Technologies. Annu. Rev. Genet. *55*, 161–181.

Lim, H., Jun, S., Park, M., Lim, J., Jeong, J., Lee, J.H., and Bang, D. (2020). Multiplex Generation, Tracking, and Functional Screening of Substitution Mutants Using a CRISPR/Retron System. ACS Synth. Biol. *9*, 1003–1009.

Lin, W., Xin, H., Zhang, Y., Wu, X., Yuan, F., and Wang, Z. (1999). The human REV1 gene codes for a DNA template-dependent dCMP transferase. Nucleic Acids Res. *27*, 4468–4475.

Lin, S., Staahl, B.T., Alla, R.K., and Doudna, J.A. (2014). Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery. eLife *3*, e04766.

Lin, Q., Zong, Y., Xue, C., Wang, S., Jin, S., Zhu, Z., Wang, Y., Anzalone, A.V., Raguram, A., Doman, J.L., et al. (2020). Prime genome editing in rice and wheat. Nat. Biotechnol. *38*, 582–585.

Lin, Q., Jin, S., Zong, Y., Yu, H., Zhu, Z., Liu, G., Kou, L., Wang, Y., Qiu, J.L., Li, J., and Gao, C. (2021). High-efficiency prime editing with optimized, paired pegRNAs in plants. Nat. Biotechnol. *39*, 923–927.

Lindahl, T. (2000). Suppression of spontaneous mutagenesis in human cells by DNA base excision-repair. Mutat. Res. *462*, 129–135.

Ling, X., Xie, B., Gao, X., Chang, L., Zheng, W., Chen, H., Huang, Y., Tan, L., Li, M., and Liu, T. (2020). Improving the efficiency of precise genome editing with site-specific Cas9-oligonucleotide conjugates. Sci. Adv. *6*, z0051.

Liu, J.J., Orlova, N., Oakes, B.L., Ma, E., Spinner, H.B., Baney, K.L.M., Chuck, J., Tan, D., Knott, G.J., Harrington, L.B., et al. (2019). CasX enzymes comprise a distinct family of RNA-guided genome editors. Nature *566*, 218–223.

Liu, B., Chen, S., Rose, A., Chen, D., Cao, F., Zwinderman, M., Kiemel, D., Aïssi, M., Dekker, F.J., and Haisma, H.J. (2020a). Inhibition of histone deacetylase 1 (HDAC1) and HDAC2 enhances CRISPR/Cas9 genome editing. Nucleic Acids Res. *48*, 517–532.

Liu, Y., Li, X., He, S., Huang, S., Li, C., Chen, Y., Liu, Z., Huang, X., and Wang, X. (2020b). Efficient generation of mouse models with the prime editing system. Cell Discov. *6*, 27.

Liu, M., Zhang, W., Xin, C., Yin, J., Shang, Y., Ai, C., Li, J., Meng, F.-L., and Hu, J. (2021a). Global detection of DNA repair outcomes induced by CRISPR-Cas9. Nucleic Acids Res. *49*, 8732–8742.

Liu, P., Liang, S.-Q., Zheng, C., Mintzer, E., Zhao, Y.G., Ponnienselvan, K., Mir, A., Sontheimer, E.J., Gao, G., Flotte, T.R., et al. (2021b). Improved prime editors enable pathogenic allele correction and cancer modelling in adult mice. Nat. Commun. *12*, 2121.

Liu, Y., Yang, G., Huang, S., Li, X., Wang, X., Li, G., Chi, T., Chen, Y., Huang, X., and Wang, X. (2021c). Enhancing prime editing by Csy4-mediated processing of pegRNA. Cell Res. *31*, 1134–1136.

Liu, G., Lin, Q., Jin, S., and Gao, C. (2022). The CRISPR-Cas toolbox and gene editing technologies. Mol. Cell *82*, 332–346.

Llorente, B., Smith, C.E., and Symington, L.S. (2008). Break-induced replication: what is it and what is it for? Cell Cycle *7*, 859–864.

Lomova, A., Clark, D.N., Campo-Fernandez, B., Flores-Bjurström, C., Kaufman, M.L., Fitz-Gibbon, S., Wang, X., Miyahira, E.Y., Brown, D., DeWitt, M.A., et al. (2019). Improving Gene Editing Outcomes in Human Hematopoietic Stem and Progenitor Cells by Temporal Control of DNA Repair. Stem Cells *37*, 284–294.

Lu, Y., Xue, J., Deng, T., Zhou, X., Yu, K., Deng, L., Huang, M., Yi, X., Liang, M., Wang, Y., et al. (2020). Safety and feasibility of CRISPR-edited T cells in patients with refractory non-small-cell lung cancer. Nat. Med. *26*, 732–740.

Ly, P., and Cleveland, D.W. (2017). Rebuilding Chromosomes After Catastrophe: Emerging Mechanisms of Chromothripsis. Trends Cell Biol. *27*, 917–930.

Ma, Y., Zhang, J., Yin, W., Zhang, Z., Song, Y., and Chang, X. (2016). Targeted AID-mediated mutagenesis (TAM) enables efficient genomic diversification in mammalian cells. Nat. Methods *13*, 1029–1035.

Ma, H., Marti-Gutierrez, N., Park, S.-W., Wu, J., Lee, Y., Suzuki, K., Koski, A., Ji, D., Hayama, T., Ahmed, R., et al. (2017a). Correction of a pathogenic gene mutation in human embryos. Nature *548*, 413–419.

Ma, M., Zhuang, F., Hu, X., Wang, B., Wen, X.Z., Ji, J.F., and Xi, J.J. (2017b). Efficient generation of mice carrying homozygous double-floxp alleles using the Cas9-Avidin/Biotin-donor DNA system. Cell Res. *27*, 578–581.

Ma, H., Marti-Gutierrez, N., Park, S.-W., Wu, J., Hayama, T., Darby, H., Van Dyken, C., Li, Y., Koski, A., Liang, D., et al. (2018). Ma et al. reply. Nature *560*, E10–E23.

Ma, L., Ruan, J., Song, J., Wen, L., Yang, D., Zhao, J., Xia, X., Chen, Y.E., Zhang, J., and Xu, J. (2020). MiCas9 increases large size gene knock-in rates and reduces undesirable on-target and off-target indel edits. Nat. Commun. *11*, 6082.

Maddalo, D., Manchado, E., Concepcion, C.P., Bonetti, C., Vidigal, J.A., Han, Y.C., Ogrodowski, P., Crippa, A., Rekhtman, N., de Stanchina, E., et al. (2014). In vivo engineering of oncogenic chromosomal rearrangements with the CRISPR/Cas9 system. Nature *516*, 423–427.

Maeder, M.L., Stefanidakis, M., Wilson, C.J., Baral, R., Barrera, L.A., Bounoutas, G.S., Bumcrot, D., Chao, H., Ciulla, D.M., DaSilva, J.A., et al. (2019). Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. Nat. Med. *25*, 229–233.

Maizels, N., and Davis, L. (2018). Initiation of homologous recombination at DNA nicks. Nucleic Acids Res. *46*, 6962–6973.

Makarova, K.S., Wolf, Y.I., Iranzo, J., Shmakov, S.A., Alkhnbashi, O.S., Brouns, S.J.J., Charpentier, E., Cheng, D., Haft, D.H., Horvath, P., et al. (2020). Evolutionary classification of CRISPR-Cas systems: a burst of class 2 and derived variants. Nat. Rev. Microbiol. *18*, 67–83.

Mali, P., Aach, J., Stranges, P.B., Esvelt, K.M., Moosburner, M., Kosuri, S., Yang, L., and Church, G.M. (2013). CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. Nat. Biotechnol. *31*, 833–838.

Malkova, A., and Haber, J.E. (2012). Mutations arising during repair of chromosome breaks. Annu. Rev. Genet. *46*, 455–473.

Mao, Z., Bozzella, M., Seluanov, A., and Gorbunova, V. (2008). Comparison of nonhomologous end joining and homologous recombination in human cells. DNA Repair (Amst.) *7*, 1765–1771.

Marquart, K.F., Allam, A., Janjuha, S., Sintsova, A., Villiger, L., Frey, N., Krauthammer, M., and Schwank, G. (2021). Predicting base editing outcomes with an attention-based deep learning algorithm trained on high-throughput target library screens. Nat. Commun. *12*, 5114.

Martín-Alonso, S., Frutos-Beltrán, E., and Menéndez-Arias, L. (2021). Reverse Transcriptase: From Transcriptomics to Genome Editing. Trends Biotechnol. *39*, 194–210.

Maruyama, T., Dougan, S.K., Truttmann, M.C., Bilate, A.M., Ingram, J.R., and Ploegh, H.L. (2015). Increasing the efficiency of precise genome editing with CRISPR-Cas9 by inhibition of nonhomologous end joining. Nat. Biotechnol. *33*, 538–542.

Mateos-Gomez, P.A., Gong, F., Nair, N., Miller, K.M., Lazzerini-Denchi, E., and Sfeir, A. (2015). Mammalian polymerase θ promotes alternative NHEJ and suppresses recombination. Nature *518*, 254–257.

Mateos-Gomez, P.A., Kent, T., Deng, S.K., McDevitt, S., Kashkina, E., Hoang, T.M., Pomerantz, R.T., and Sfeir, A. (2017). The helicase domain of Polθ counteracts RPA to promote alt-NHEJ. Nat. Struct. Mol. Biol. *24*, 1116–1123.

Matos, J., and West, S.C. (2014). Holliday junction resolution: regulation in space and time. DNA Repair (Amst.) *19*, 176–181.

Maurissen, T.L., and Woltjen, K. (2020). Synergistic gene editing in human iPS cells via cell cycle and DNA repair modulation. Nat. Commun. *11*, 2876.

Mazina, O.M., Keskin, H., Hanamshet, K., Storici, F., and Mazin, A.V. (2017). Rad52 Inverse Strand Exchange Drives RNA-Templated DNA Double-Strand Break Repair. Mol. Cell *67*, 19–29.e3.

McVey, M., and Lee, S.E. (2008). MMEJ repair of double-strand breaks (director's cut): deleted sequences and alternative endings. Trends Genet. *24*, 529–538.

McVey, M., Khodaverdian, V.Y., Meyer, D., Cerqueira, P.G., and Heyer, W.D. (2016). Eukaryotic DNA Polymerases in Homologous Recombination. Annu. Rev. Genet. *50*, 393–421.

Mercer, A.C., Gaj, T., Fuller, R.P., and Barbas, C.F., 3rd (2012). Chimeric TALE recombinases with programmable DNA sequence specificity. Nucleic Acids Res. *40*, 11163–11172.

Merrick, C.A., Zhao, J., and Rosser, S.J. (2018). Serine Integrases: Advancing Synthetic Biology. ACS Synth. Biol. *7*, 299–310.

Millman, A., Bernheim, A., Stokar-Avihail, A., Fedorenko, T., Voichek, M., Leavitt, A., Oppenheimer-Shaanan, Y., and Sorek, R. (2020). Bacterial Retrons Function In Anti-Phage Defense. Cell *183*, 1551–1561.e12.

Mimitou, E.P., and Symington, L.S. (2010). Ku prevents Exo1 and Sgs1-dependent resection of DNA ends in the absence of a functional MRX complex or Sae2. EMBO J. *29*, 3358–3369.

Min, Y.L., Li, H., Rodriguez-Caycedo, C., Mireault, A.A., Huang, J., Shelton, J.M., McAnally, J.R., Amoasii, L., Mammen, P.P.A., Bassel-Duby, R., and Olson, E.N. (2019). CRISPR-Cas9 corrects Duchenne muscular dystrophy exon 44 deletion mutations in mice and human cells. Sci. Adv. *5*, v4324.

Miyaoka, Y., Chan, A.H., Judge, L.M., Yoo, J., Huang, M., Nguyen, T.D., Lizarraga, P.P., So, P.L., and Conklin, B.R. (2014). Isolation of single-base genome-edited human iPS cells without antibiotic selection. Nat. Methods *11*, 291–293.

Mohni, K.N., Wessel, S.R., Zhao, R., Wojciechowski, A.C., Luzwick, J.W., Layden, H., Eichman, B.F., Thompson, P.S., Mehta, K.P.M., and Cortez, D. (2019). HMCES Maintains Genome Integrity by Shielding Abasic Sites in Single-Strand DNA. Cell *176*, 144–153.e13.

Mok, B.Y., de Moraes, M.H., Zeng, J., Bosch, D.E., Kotrys, A.V., Raguram, A., Hsu, F., Radey, M.C., Peterson, S.B., Mootha, V.K., et al. (2020). A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing. Nature *583*, 631–637.

Molla, K.A., and Yang, Y. (2019). CRISPR/Cas-Mediated Base Editing: Technical Considerations and Practical Applications. Trends Biotechnol. *37*, 1121–1142.

Monteys, A.M., Ebanks, S.A., Keiser, M.S., and Davidson, B.L. (2017). CRISPR/Cas9 Editing of the Mutant Huntingtin Allele In Vitro and In Vivo. Mol. Ther. *25*, 12–23.

Mosbach, V., Poggi, L., and Richard, G.-F. (2019). Trinucleotide repeat instability during double-strand break repair: from mechanisms to gene therapy. Curr. Genet. *65*, 17–28.

Moscou, M.J., and Bogdanove, A.J. (2009). A simple cipher governs DNA recognition by TAL effectors. Science *326*, 1501.

Mullard, A. (2020). Gene-editing pipeline takes off. Nat. Rev. Drug Discov. *19*, 367–372.

Muñoz-López, M., and García-Pérez, J.L. (2010). DNA transposons: nature and applications in genomics. Curr. Genomics *11*, 115–128.

Murphy, M.P., and Hartley, R.C. (2018). Mitochondria as a therapeutic target for common pathologies. Nat. Rev. Drug Discov. *17*, 865–886.

Musunuru, K., Chadwick, A.C., Mizoguchi, T., Garcia, S.P., DeNizio, J.E., Reiss, C.W., Wang, K., Iyer, S., Dutta, C., Clendaniel, V., et al. (2021). In vivo CRISPR base editing of PCSK9 durably lowers cholesterol in primates. Nature *593*, 429–434.

Nabel, C.S., Manning, S.A., and Kohli, R.M. (2012). The curious chemical biology of cytosine: deamination, methylation, and oxidation as modulators of genomic potential. ACS Chem. Biol. *7*, 20–30.

Nakade, S., Tsubota, T., Sakane, Y., Kume, S., Sakamoto, N., Obara, M., Daimon, T., Sezutsu, H., Yamamoto, T., Sakuma, T., and Suzuki, K.T. (2014). Microhomology-mediated end-joining-dependent integration of donor DNA in cells and animals using TALENs and CRISPR/Cas9. Nat. Commun. *5*, 5560.

Nakajima, K., Zhou, Y., Tomita, A., Hirade, Y., Gurumurthy, C.B., and Nakada, S. (2018). Precise and efficient nucleotide substitution near genomic nick via noncanonical homology-directed repair. Genome Res. *28*, 223–230.

Nambiar, T.S., Billon, P., Diedenhofen, G., Hayward, S.B., Taglialatela, A., Cai, K., Huang, J.W., Leuzzi, G., Cuella-Martin, R., Palacios, A., et al. (2019). Stimulation of CRISPR-mediated homology-directed repair by an engineered RAD18 variant. Nat. Commun. *10*, 3395.

Nami, F., Basiri, M., Satarian, L., Curtiss, C., Baharvand, H., and Verfaillie, C. (2018). Strategies for In Vivo Genome Editing in Nondividing Cells. Trends Biotechnol. *36*, 770–786.

Natsume, T., Kiyomitsu, T., Saga, Y., and Kanemaki, M.T. (2016). Rapid Protein Depletion in Human Cells by Auxin-Inducible Degron Tagging with Short Homology Donors. Cell Rep. *15*, 210–218.

Nelson, J.R., Lawrence, C.W., and Hinkle, D.C. (1996). Deoxycytidyl transferase activity of yeast REV1 protein. Nature *382*, 729–731.

Nelson, J.W., Randolph, P.B., Shen, S.P., Everette, K.A., Chen, P.J., Anzalone, A.V., An, M., Newby, G.A., Chen, J.C., Hsu, A., et al. (2021). Engineered pegRNAs improve prime editing efficiency. Nat. Biotechnol. Published online October 4, 2021. https://doi.org/10.1038/s41587-021-01039-7.

Newby, G.A., and Liu, D.R. (2021). In vivo somatic cell base editing and prime editing. Mol. Ther. *29*, 3107–3124.

Newby, G.A., Yen, J.S., Woodard, K.J., Mayuranathan, T., Lazzarotto, C.R., Li, Y., Sheppard-Tillman, H., Porter, S.N., Yao, Y., Mayberry, K., et al. (2021). Base editing of haematopoietic stem cells rescues sickle cell disease in mice. Nature *595*, 295–302.

Nguyen, D.N., Roth, T.L., Li, P.J., Chen, P.A., Apathy, R., Mamedov, M.R., Vo, L.T., Tobin, V.R., Goodman, D., Shifrut, E., et al. (2020). Polymer-stabilized Cas9 nanoparticles and modified repair templates increase genome editing efficiency. Nat. Biotechnol. *38*, 44–49.

Nik-Zainal, S., Alexandrov, L.B., Wedge, D.C., Van Loo, P., Greenman, C.D., Raine, K., Jones, D., Hinton, J., Marshall, J., Stebbings, L.A., et al.; Breast Cancer Working Group of the International Cancer Genome Consortium (2012). Mutational processes molding the genomes of 21 breast cancers. Cell *149*, 979–993.

Nishida, K., Arazoe, T., Yachie, N., Banno, S., Kakimoto, M., Tabata, M., Mochizuki, M., Miyabe, A., Araki, M., Hara, K.Y., et al. (2016). Targeted nucleotide

editing using hybrid prokaryotic and vertebrate adaptive immune systems. Science *353*, aaf8729.

Nishimasu, H., Ran, F.A., Hsu, P.D., Konermann, S., Shehata, S.I., Dohmae, N., Ishitani, R., Zhang, F., and Nureki, O. (2014). Crystal structure of Cas9 in complex with guide RNA and target DNA. Cell *156*, 935–949.

Niu, Y., Shen, B., Cui, Y., Chen, Y., Wang, J., Wang, L., Kang, Y., Zhao, X., Si, W., Li, W., et al. (2014). Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. Cell *156*, 836–843.

Nussenzweig, P.M., and Marraffini, L.A. (2020). Molecular Mechanisms of CRISPR-Cas Immunity in Bacteria. Annu. Rev. Genet. *54*, 93–120.

Ochs, F., Somyajit, K., Altmeyer, M., Rask, M.B., Lukas, J., and Lukas, C. (2016). 53BP1 fosters fidelity of homology-directed DNA repair. Nat. Struct. Mol. Biol. *23*, 714–721.

Olivieri, M., Cho, T., Álvarez-Quilón, A., Li, K., Schellenberg, M.J., Zimmermann, M., Hustedt, N., Rossi, S.E., Adam, S., Melo, H., et al. (2020). A Genetic Map of the Response to DNA Damage in Human Cells. Cell *182*, 481–496.e21.

Olorunniji, F.J., Rosser, S.J., and Stark, W.M. (2016). Site-specific recombinases: molecular machines for the Genetic Revolution. Biochem. J. *473*, 673–684.

Orthwein, A., Noordermeer, S.M., Wilson, M.D., Landry, S., Enchev, R.I., Sherker, A., Munro, M., Pinder, J., Salsman, J., Dellaire, G., et al. (2015). A mechanism for the suppression of homologous recombination in G1 cells. Nature *528*, 422–426.

Ouellet, D.L., Cherif, K., Rousseau, J., and Tremblay, J.P. (2017). Deletion of the GAA repeats from the human frataxin gene using the CRISPR-Cas9 system in YG8R-derived cells and mouse models of Friedreich ataxia. Gene Ther. *24*, 265–274.

Owens, D.D.G., Caulder, A., Frontera, V., Harman, J.R., Allan, A.J., Bucakci, A., Greder, L., Codner, G.F., Hublitz, P., McHugh, P.J., et al. (2019). Microhomologies are prevalent at Cas9-induced larger deletions. Nucleic Acids Res. *47*, 7402–7417.

Paix, A., Folkmann, A., Goldman, D.H., Kulaga, H., Grzelak, M.J., Rasoloson, D., Paidemarry, S., Green, R., Reed, R.R., and Seydoux, G. (2017). Precision genome editing using synthesis-dependent repair of Cas9-induced DNA breaks. Proc. Natl. Acad. Sci. USA *114*, E10745–E10754.

Pallarès-Masmitjà, M., Ivančić, D., Mir-Pedrol, J., Jaraba-Wallace, J., Tagliani, T., Oliva, B., Rahmeh, A., Sánchez-Mejías, A., and Güell, M. (2021). Find and cut-and-transfer (FiCAT) mammalian genome engineering. Nat. Commun. *12*, 7071.

Papathanasiou, S., Markoulaki, S., Blaine, L.J., Leibowitz, M.L., Zhang, C.-Z., Jaenisch, R., and Pellman, D. (2021). Whole chromosome loss and genomic instability in mouse embryos after CRISPR-Cas9 genome editing. Nat. Commun. *12*, 5855.

Park, J.-U., Tsai, A.W.-L., Mehrotra, E., Petassi, M.T., Hsieh, S.-C., Ke, A., Peters, J.E., and Kellogg, E.H. (2021). Structural basis for target site selection in RNA-guided DNA transposition systems. Science *373*, 768–774.

Paulis, M., Castelli, A., Lizier, M., Susani, L., Lucchini, F., Villa, A., and Vezzoni, P. (2015). A pre-screening FISH-based method to detect CRISPR/Cas9 off-targets in mouse embryonic stem cells. Sci. Rep. *5*, 12327.

Paulsen, B.S., Mandal, P.K., Frock, R.L., Boyraz, B., Yadav, R., Upadhyayula, S., Gutierrez-Martinez, P., Ebina, W., Fasth, A., Kirchhausen, T., et al. (2017). Ectopic expression of RAD52 and dn53BP1 improves homology-directed repair during CRISPR-Cas9 genome editing. Nat. Biomed. Eng. *1*, 878–888.

Pausch, P., Al-Shayeb, B., Bisom-Rapp, E., Tsuchida, C.A., Li, Z., Cress, B.F., Knott, G.J., Jacobsen, S.E., Banfield, J.F., and Doudna, J.A. (2020). CRISPR-CasΦ from huge phages is a hypercompact genome. Science *369*, 333–337.

Petassi, M.T., Hsieh, S.C., and Peters, J.E. (2020). Guide RNA Categorization Enables Target Site Choice in Tn7-CRISPR-Cas Transposons. Cell *183*, 1757–1771.e18.

Peterka, M., Akrap, N., Li, S., Wimberger, S., Hsieh, P.-P., Degtev, D., Bestas, B., Barr, J., van de Plassche, S., Mendoza-Garcia, P., et al. (2021). Harnessing DSB repair to promote efficient homology-dependent and -independent prime editing. bioRxiv. https://doi.org/10.1101/2021.08.10.455572.

Peters, J.E., Makarova, K.S., Shmakov, S., and Koonin, E.V. (2017). Recruitment of CRISPR-Cas systems by Tn7-like transposons. Proc. Natl. Acad. Sci. USA *114*, E7358–E7366.

Petri, K., Zhang, W., Ma, J., Schmidts, A., Lee, H., Horng, J.E., Kim, D.Y., Kurt, I.C., Clement, K., Hsu, J.Y., et al. (2021). CRISPR prime editing with ribonucleoprotein complexes in zebrafish and primary human cells. Nat. Biotechnol. Published online April 29, 2021. https://doi.org/10.1038/s41587-021-00901-y.

Pham, N., Yan, Z., Yu, Y., Faria Afreen, M., Malkova, A., Haber, J.E., and Ira, G. (2021). Mechanisms restraining break-induced replication at two-ended DNA double-strand breaks. EMBO J. *40*, e104847.

Piazza, A., Wright, W.D., and Heyer, W.D. (2017). Multi-invasions Are Recombination Byproducts that Induce Chromosomal Rearrangements. Cell *170*, 760–773.e15.

Pickar-Oliver, A., and Gersbach, C.A. (2019). The next generation of CRISPR-Cas technologies and applications. Nat. Rev. Mol. Cell Biol. *20*, 490–507.

Pinder, J., Salsman, J., and Dellaire, G. (2015). Nuclear domain 'knock-in' screen for the evaluation and identification of small molecule enhancers of CRISPR-based genome editing. Nucleic Acids Res. *43*, 9379–9392.

Porteus, M.H. (2019). A New Class of Medicines through DNA Editing. N. Engl. J. Med. *380*, 947–959.

Porto, E.M., Komor, A.C., Slaymaker, I.M., and Yeo, G.W. (2020). Base editing: advances and therapeutic opportunities. Nat. Rev. Drug Discov. *19*, 839–859.

Prasad, R., Singhal, R.K., Srivastava, D.K., Molina, J.T., Tomkinson, A.E., and Wilson, S.H. (1996). Specific interaction of DNA polymerase beta and DNA ligase I in a multiprotein base excision repair complex from bovine testis. J. Biol. Chem. *271*, 16000–16007.

Prasad, R., Lavrik, O.I., Kim, S.J., Kedar, P., Yang, X.P., Vande Berg, B.J., and Wilson, S.H. (2001). DNA polymerase beta -mediated long patch base excision repair. Poly(ADP-ribose)polymerase-1 stimulates strand displacement DNA synthesis. J. Biol. Chem. *276*, 32411–32414.

Pristyazhnyuk, I.E., Minina, J., Korablev, A., Serova, I., Fishman, V., Gridina, M., Rozhdestvensky, T.S., Gubar, L., Skryabin, B.V., and Serov, O.L. (2019). Time origin and structural analysis of the induced CRISPR/cas9 megabase-sized deletions and duplications involving the Cntn6 gene in mice. Sci. Rep. *9*, 14161.

Provenzano, C., Cappella, M., Valaperta, R., Cardani, R., Meola, G., Martelli, F., Cardinali, B., and Falcone, G. (2017). CRISPR/Cas9-Mediated Deletion of CTG Expansions Recovers Normal Phenotype in Myogenic Cells Derived from Myotonic Dystrophy 1 Patients. Mol. Ther. Nucleic Acids *9*, 337–348.

Qi, L.S., Larson, M.H., Gilbert, L.A., Doudna, J.A., Weissman, J.S., Arkin, A.P., and Lim, W.A. (2013). Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell *152*, 1173–1183.

Querques, I., Schmitz, M., Oberli, S., Chanez, C., and Jinek, M. (2021). Target site selection and remodelling by type V CRISPR-transposon systems. Nature *599*, 497–502.

Quinn, J., Musa, A., Kantor, A., McClements, M.E., Cehajic-Kapetanovic, J., MacLaren, R.E., and Xue, K. (2021). Genome-Editing Strategies for Treating Human Retinal Degenerations. Hum. Gene Ther. *32*, 247–259.

Ramsden, D.A., and Nussenzweig, A. (2021). Mechanisms driving chromosomal translocations: lost in time and space. Oncogene *40*, 4263–4270.

Ramsden, D.A., Carvajal-Garcia, J., and Gupta, G.P. (2021). Mechanism, cellular functions and cancer roles of polymerase-theta-mediated DNA end joining. Nat. Rev. Mol. Cell Biol. Published online September 14, 2021. https://doi.org/10.1038/s41580-021-00405-2.

Ran, F.A., Hsu, P.D., Lin, C.Y., Gootenberg, J.S., Konermann, S., Trevino, A.E., Scott, D.A., Inoue, A., Matoba, S., Zhang, Y., and Zhang, F. (2013). Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. Cell *154*, 1380–1389.

Rausch, T., Jones, D.T., Zapatka, M., Stütz, A.M., Zichner, T., Weischenfeldt, J., Jäger, N., Remke, M., Shih, D., Northcott, P.A., et al. (2012). Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. Cell *148*, 59–71.

Rebollo, R., Romanish, M.T., and Mager, D.L. (2012). Transposable elements: an abundant and natural source of regulatory sequences for host genes. Annu. Rev. Genet. *46*, 21–42.

Rees, H.A., and Liu, D.R. (2018). Base editing: precision chemistry on the genome and transcriptome of living cells. Nat. Rev. Genet. *19*, 770–788.

Rees, H.A., Yeh, W.H., and Liu, D.R. (2019). Development of hRad51-Cas9 nickase fusions that mediate HDR without double-stranded breaks. Nat. Commun. *10*, 2212.

Reginato, G., and Cejka, P. (2020). The MRE11 complex: A versatile toolkit for the repair of broken DNA. DNA Repair (Amst.) *91-92*, 102869.

Reginato, G., Cannavo, E., and Cejka, P. (2017). Physiological protein blocks direct the Mre11-Rad50-Xrs2 and Sae2 nuclease complex to initiate DNA end resection. Genes Dev. *31*, 2325–2330.

Reint, G., Li, Z., Labun, K., Keskitalo, S., Soppa, I., Mamia, K., Tolo, E., Szymanska, M., Meza-Zepeda, L.A., Lorenz, S., et al. (2021). Rapid genome editing by CRISPR-Cas9-POLD3 fusion. eLife *10*, e75415.

Renaud, J.B., Boix, C., Charpentier, M., De Cian, A., Cochennec, J., Duvernois-Berthet, E., Perrouault, L., Tesson, L., Edouard, J., Thinard, R., et al. (2016). Improved Genome Editing Efficiency and Flexibility Using Modified Oligonucleotides with TALEN and CRISPR-Cas9 Nucleases. Cell Rep. *14*, 2263–2272.

Richardson, C.D., Ray, G.J., DeWitt, M.A., Curie, G.L., and Corn, J.E. (2016). Enhancing homology-directed genome editing by catalytically active and inactive CRISPR-Cas9 using asymmetric donor DNA. Nat. Biotechnol. *34*, 339–344.

Richardson, C.D., Kazane, K.R., Feng, S.J., Zelin, E., Bray, N.L., Schäfer, A.J., Floor, S.N., and Corn, J.E. (2018). CRISPR-Cas9 genome editing in human cells occurs via the Fanconi anemia pathway. Nat. Genet. *50*, 1132–1139.

Richardson, R.R., Steyert, M., Inen, J., Khim, S., Romanowski, A.J., Altas, B., and Poulopoulos, A. (2020). Cas9 fusions for precision in vivo editing. bioRxiv. https://doi.org/10.1101/2020.07.15.199620.

Richter, M.F., Zhao, K.T., Eton, E., Lapinaite, A., Newby, G.A., Thuronyi, B.W., Wilson, C., Koblan, L.W., Zeng, J., Bauer, D.E., et al. (2020). Phage-assisted evolution of an adenine base editor with improved Cas domain compatibility and activity. Nat. Biotechnol. *38*, 883–891.

Riesenberg, S., and Maricic, T. (2018). Targeting repair pathways with small molecules increases precise genome editing in pluripotent stem cells. Nat. Commun. *9*, 2164.

Riesenberg, S., Chintalapati, M., Macak, D., Kanis, P., Maricic, T., and Pääbo, S. (2019). Simultaneous precise editing of multiple genes in human cells. Nucleic Acids Res. *47*, e116.

Rivera-Torres, N., Banas, K., Bialk, P., Bloh, K.M., and Kmiec, E.B. (2017). Insertional Mutagenesis by CRISPR/Cas9 Ribonucleoprotein Gene Editing in Cells Targeted for Point Mutation Repair Directed by Short Single-Stranded DNA Oligonucleotides. PLoS ONE *12*, e0169350.

Robert, F., Barbeau, M., Éthier, S., Dostie, J., and Pelletier, J. (2015). Pharmacological inhibition of DNA-PK stimulates Cas9-mediated genome editing. Genome Med. *7*, 93.

Roberts, S.A., and Gordenin, D.A. (2014). Hypermutation in human cancer genomes: footprints and mechanisms. Nat. Rev. Cancer *14*, 786–800.

Roberts, B., Haupt, A., Tucker, A., Grancharova, T., Arakaki, J., Fuqua, M.A., Nelson, A., Hookway, C., Ludmann, S.A., Mueller, I.A., et al. (2017). Systematic gene tagging using CRISPR/Cas9 in human stem cells to illuminate cell organization. Mol. Biol. Cell *28*, 2854–2874.

Rosello, M., Vougny, J., Czarny, F., Mione, M.C., Concordet, J.P., Albadri, S., and Del Bene, F. (2021). Precise base editing for the *in vivo* study of developmental signaling and human pathologies in zebrafish. eLife *10*, e65552.

Rossidis, A.C., Stratigis, J.D., Chadwick, A.C., Hartman, H.A., Ahn, N.J., Li, H., Singh, K., Coons, B.E., Li, L., Lv, W., et al. (2018). In utero CRISPR-mediated therapeutic editing of metabolic genes. Nat. Med. *24*, 1513–1518.

Roth, T.L., Li, P.J., Blaeschke, F., Nies, J.F., Apathy, R., Mowery, C., Yu, R., Nguyen, M.L.T., Lee, Y., Truong, A., et al. (2020). Pooled Knockin Targeting for Genome Engineering of Cellular Immunotherapies. Cell *181*, 728–744.e21.

Rothgangl, T., Dennis, M.K., Lin, P.J.C., Oka, R., Witzigmann, D., Villiger, L., Qi, W., Hruzova, M., Kissling, L., Lenggenhager, D., et al. (2021). In vivo adenine base editing of PCSK9 in macaques reduces LDL cholesterol levels. Nat. Biotechnol. *39*, 949–957.

Rouet, P., Smih, F., and Jasin, M. (1994). Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease. Mol. Cell. Biol. *14*, 8096–8106.

Rousset, F., Dowding, J., Bernheim, A., Rocha, E.P.C., and Bikard, D. (2021). Prophage-encoded hotspots of bacterial immune systems. bioRxiv. https://doi.org/10.1101/2021.01.21.427644.

Rubin, B.E., Diamond, S., Cress, B.F., Crits-Christoph, A., Lou, Y.C., Borges, A.L., Shivram, H., He, C., Xu, M., Zhou, Z., et al. (2021). Species- and site-specific genome editing in complex bacterial communities. Nat. Microbiol. Published online December 6, 2021. https://doi.org/10.1038/s41564-021-01014-7.

Ryu, S.M., Koo, T., Kim, K., Lim, K., Baek, G., Kim, S.T., Kim, H.S., Kim, D.E., Lee, H., Chung, E., and Kim, J.S. (2018). Adenine base editing in mouse embryos and an adult mouse model of Duchenne muscular dystrophy. Nat. Biotechnol. *36*, 536–539.

Sabharwal, A., Kar, B., Restrepo-Castillo, S., Holmberg, S.R., Mathew, N.D., Kendall, B.L., Cotter, R.P., WareJoncas, Z., Seiler, C., Nakamaru-Ogiso, E., et al. (2021). The FusX TALE Base Editor (FusXTBE) for Rapid Mitochondrial DNA Programming of Human Cells *In Vitro* and Zebrafish Disease Models *In Vivo*. CRISPR J *4*, 799–821.

Saito, M., Ladha, A., Strecker, J., Faure, G., Neumann, E., Altae-Tran, H., Macrae, R.K., and Zhang, F. (2021). Dual modes of CRISPR-associated transposon homing. Cell *184*, 2441–2453.e18.

Sakata, R.C., Ishiguro, S., Mori, H., Tanaka, M., Tatsuno, K., Ueda, H., Yamamoto, S., Seki, M., Masuyama, N., Nishida, K., et al. (2020). Base editors for simultaneous introduction of C-to-T and A-to-G mutations. Nat. Biotechnol. *38*, 865–869.

Sanders, M.A., Vöhringer, H., Forster, V.J., Moore, L., Campbell, B.B., Hooks, Y., Edwards, M., Bianchi, V., Coorens, T.H.H., Butler, T.M., et al. (2021). Life without mismatch repair. bioRxiv. https://doi.org/10.1101/2021.04.14.437578.

Sangree, A.K., Griffith, A.L., Szegletes, Z.M., Roy, P., DeWeirdt, P.C., Hegde, M., McGee, A.V., Hanna, R.E., and Doench, J.G. (2021). Benchmarking of SpCas9 variants enables deeper base editor screens of BRCA1 and BCL2. bioRxiv. https://doi.org/10.1101/2021.08.18.456848.

Savic, N., Ringnalda, F.C., Lindsay, H., Berk, C., Bargsten, K., Li, Y., Neri, D., Robinson, M.D., Ciaudo, C., Hall, J., et al. (2018). Covalent linkage of the DNA repair template to the CRISPR-Cas9 nuclease enhances homology-directed repair. eLife *7*, e33761.

Schanz, S., Castor, D., Fischer, F., and Jiricny, J. (2009). Interference of mismatch and base excision repair during the processing of adjacent U/G mispairs may play a key role in somatic hypermutation. Proc. Natl. Acad. Sci. USA *106*, 5593–5598.

Schene, I.F., Joore, I.P., Oka, R., Mokry, M., van Vugt, A.H.M., van Boxtel, R., van der Doef, H.P.J., van der Laan, L.J.W., Verstegen, M.M.A., van Hasselt, P.M., et al. (2020). Prime editing for functional repair in patient-derived disease models. Nat. Commun. *11*, 5352.

Schep, R., Brinkman, E.K., Leemans, C., Vergara, X., van der Weide, R.H., Morris, B., van Schaik, T., Manzo, S.G., Peric-Hupkes, D., van den Berg, J., et al. (2021). Impact of chromatin context on Cas9-induced DNA double-strand break repair pathway balance. Mol. Cell *81*, 2216–2230.e10.

Schiroli, G., Conti, A., Ferrari, S., Della Volpe, L., Jacob, A., Albano, L., Beretta, S., Calabria, A., Vavassori, V., Gasparini, P., et al. (2019). Precise Gene Editing Preserves Hematopoietic Stem Cell Function following Transient p53-Mediated DNA Damage Response. Cell Stem Cell *24*, 551–565.e8.

Schmid-Burgk, J.L., Höning, K., Ebert, T.S., and Hornung, V. (2016). CRISPaint allows modular base-specific gene tagging using a ligase-4-dependent mechanism. Nat. Commun. *7*, 12338.

Schneider, A. (2020). A short history of guide RNAs: The intricate path that led to the discovery of a basic biological concept. EMBO Rep. *21*, e51918.

Schubert, M.G., Goodman, D.B., Wannier, T.M., Kaur, D., Farzadfard, F., Lu, T.K., Shipman, S.L., and Church, G.M. (2021). High-throughput functional variant screens via in vivo production of single-stranded DNA. Proc. Natl. Acad. Sci. USA *118*, e2018181118.

Scully, R., Panday, A., Elango, R., and Willis, N.A. (2019). DNA double-strand break repair-pathway choice in somatic mammalian cells. Nat. Rev. Mol. Cell Biol. *20*, 698–714.

Sertic, S., Quadri, R., Lazzaro, F., and Muzi-Falconi, M. (2020). EXO1: A tightly regulated nuclease. DNA Repair (Amst.) *93*, 102929.

Sfeir, A., and Symington, L.S. (2015). Microhomology-Mediated End Joining: A Back-up Survival Mechanism or Dedicated Pathway? Trends Biochem. Sci. *40*, 701–714.

Shalem, O., Sanjana, N.E., and Zhang, F. (2015). High-throughput functional genomics using CRISPR-Cas9. Nat. Rev. Genet. *16*, 299–311.

Shao, S., Ren, C., Liu, Z., Bai, Y., Chen, Z., Wei, Z., Wang, X., Zhang, Z., and Xu, K. (2017). Enhancing CRISPR/Cas9-mediated homology-directed repair in mammalian cells by expressing Saccharomyces cerevisiae Rad52. Int. J. Biochem. Cell Biol. *92*, 43–52.

Sharon, E., Chen, S.A., Khosla, N.M., Smith, J.D., Pritchard, J.K., and Fraser, H.B. (2018). Functional Genetic Variants Revealed by Massively Parallel Precise Genome Editing. Cell *175*, 544–557.e16.

She, W., Cambouris, C.B., and Craig, N.L. (2015). Different DNA repair pathways are required following excision and integration of the DNA cut & paste transposon piggyBat in Saccharomyces cerevisiae. bioRxiv. https://doi.org/10.1101/015289.

Shen, J.P., and Ideker, T. (2017). Correcting CRISPR for copy number. Nat. Genet. *49*, 1674–1675.

Shen, M.W., Arbab, M., Hsu, J.Y., Worstell, D., Culbertson, S.J., Krabbe, O., Cassa, C.A., Liu, D.R., Gifford, D.K., and Sherwood, R.I. (2018). Predictable and precise template-free CRISPR editing of pathogenic variants. Nature *563*, 646–651.

Shen, Y., Gomez-Blanco, J., Petassi, M.T., Peters, J.E., Ortega, J., and Guarné, A. (2021). Structural basis for DNA targeting by the Tn7 transposon. bioRxiv. https://doi.org/10.1101/2021.05.24.445525.

Shin, J.W., Kim, K.H., Chao, M.J., Atwal, R.S., Gillis, T., MacDonald, M.E., Gusella, J.F., and Lee, J.M. (2016). Permanent inactivation of Huntington's disease mutation by personalized allele-specific CRISPR/Cas9. Hum. Mol. Genet. *25*, 4566–4576.

Shin, H.Y., Wang, C., Lee, H.K., Yoo, K.H., Zeng, X., Kuhns, T., Yang, C.M., Mohr, T., Liu, C., and Hennighausen, L. (2017). CRISPR/Cas9 targeting events cause complex deletions and insertions at 17 sites in the mouse genome. Nat. Commun. *8*, 15464.

Shivram, H., Cress, B.F., Knott, G.J., and Doudna, J.A. (2021). Controlling and enhancing CRISPR systems. Nat. Chem. Biol. *17*, 10–19.

Shou, J., Li, J., Liu, Y., and Wu, Q. (2018). Precise and Predictable CRISPR Chromosomal Rearrangements Reveal Principles of Cas9-Mediated Nucleotide Insertion. Mol. Cell *71*, 498–509.e4.

Shy, B.R., Vykunta, V., Ha, A., Roth, T.L., Talbot, A., Nguyen, D.N., Chen, Y.Y., Blaeschke, F., Vedova, S., Mamedov, M.R., et al. (2021). Hybrid ssDNA repair templates enable high yield genome engineering in primary cells for disease modeling and cell therapy manufacturing. bioRxiv. https://doi.org/10.1101/2021.09.02.458799.

Simon, A.J., Ellington, A.D., and Finkelstein, I.J. (2019). Retrons and their applications in genome engineering. Nucleic Acids Res. *47*, 11007–11019.

Sinha, S., Barbosa, K., Cheng, K., Leiserson, M.D.M., Jain, P., Deshpande, A., Wilson, D.M., 3rd, Ryan, B.M., Luo, J., Ronai, Z.A., et al. (2021). A systematic genome-wide mapping of oncogenic mutation selection during CRISPR-Cas9 genome editing. Nat. Commun. *12*, 6512.

Skryabin, B.V., Kummerfeld, D.M., Gubar, L., Seeger, B., Kaiser, H., Stegemann, A., Roth, J., Meuth, S.G., Pavenstädt, H., Sherwood, J., et al. (2020). Pervasive head-to-tail insertions of DNA templates mask desired CRISPR-Cas9-mediated genome editing events. Sci. Adv. *6*, x2941.

Smith, C.J., Castanon, O., Said, K., Volf, V., Khoshakhlagh, P., Hornick, A., Ferreira, R., Wu, C.T., Güell, M., Garg, S., et al. (2020). Enabling large-scale genome editing at repetitive elements by reducing DNA nicking. Nucleic Acids Res. *48*, 5183–5195.

Song, J., Yang, D., Xu, J., Zhu, T., Chen, Y.E., and Zhang, J. (2016). RS-1 enhances CRISPR/Cas9- and TALEN-mediated knock-in efficiency. Nat. Commun. *7*, 10548.

Song, C.Q., Jiang, T., Richter, M., Rhym, L.H., Koblan, L.W., Zafra, M.P., Schatoff, E.M., Doman, J.L., Cao, Y., Dow, L.E., et al. (2020). Adenine base editing in an adult mouse model of tyrosinaemia. Nat. Biomed. Eng. *4*, 125–130.

Song, M., Lim, J.M., Min, S., Oh, J.-S., Kim, D.Y., Woo, J.-S., Nishimasu, H., Cho, S.-R., Yoon, S., and Kim, H.H. (2021). Generation of a more efficient prime editor 2 by addition of the Rad51 DNA-binding domain. Nat. Commun. *12*, 5617.

Sriramachandran, A.M., Petrosino, G., Méndez-Lago, M., Schäfer, A.J., Batista-Nascimento, L.S., Zilio, N., and Ulrich, H.D. (2020). Genome-wide Nucleotide-Resolution Mapping of DNA Replication Patterns, Single-Strand Breaks, and Lesions by GLOE-Seq. Mol. Cell *78*, 975–985.e7.

Srivastava, M., Nambiar, M., Sharma, S., Karki, S.S., Goldsmith, G., Hegde, M., Kumar, S., Pandey, M., Singh, R.K., Ray, P., et al. (2012). An inhibitor of nonhomologous end-joining abrogates double-strand break repair and impedes cancer progression. Cell *151*, 1474–1487.

Stadtmauer, E.A., Fraietta, J.A., Davis, M.M., Cohen, A.D., Weber, K.L., Lancaster, E., Mangan, P.A., Kulikovskaya, I., Gupta, M., Chen, F., et al. (2020). CRISPR-engineered T cells in patients with refractory cancer. Science *367*, eaba7365.

Stella, S., Alcón, P., and Montoya, G. (2017). Class 2 CRISPR-Cas RNA-guided endonucleases: Swiss Army knives of genome editing. Nat. Struct. Mol. Biol. *24*, 882–892.

Stephens, P.J., Greenman, C.D., Fu, B., Yang, F., Bignell, G.R., Mudie, L.J., Pleasance, E.D., Lau, K.W., Beare, D., Stebbings, L.A., et al. (2011). Massive genomic rearrangement acquired in a single catastrophic event during cancer development. Cell *144*, 27–40.

Stinson, B.M., and Loparo, J.J. (2021). Repair of DNA Double-Strand Breaks by the Nonhomologous End Joining Pathway. Annu. Rev. Biochem. *90*, 137–164.

Stinson, B.M., Moreno, A.T., Walter, J.C., and Loparo, J.J. (2020). A Mechanism to Minimize Errors during Non-homologous End Joining. Mol. Cell *77*, 1080–1091.e8.

Storici, F., Snipe, J.R., Chan, G.K., Gordenin, D.A., and Resnick, M.A. (2006). Conservative repair of a chromosomal double-strand break by single-strand DNA through two steps of annealing. Mol. Cell. Biol. *26*, 7645–7657.

Strecker, J., Jones, S., Koopal, B., Schmid-Burgk, J., Zetsche, B., Gao, L., Makarova, K.S., Koonin, E.V., and Zhang, F. (2019a). Engineering of CRISPR-Cas12b for human genome editing. Nat. Commun. *10*, 212.

Strecker, J., Ladha, A., Gardner, Z., Schmid-Burgk, J.L., Makarova, K.S., Koonin, E.V., and Zhang, F. (2019b). RNA-guided DNA insertion with CRISPR-associated transposases. Science *365*, 48–53.

Suzuki, K., and Izpisua Belmonte, J.C. (2018). In vivo genome editing via the HITI method as a tool for gene therapy. J. Hum. Genet. *63*, 157–164.

Suzuki, K., Tsunekawa, Y., Hernandez-Benitez, R., Wu, J., Zhu, J., Kim, E.J., Hatanaka, F., Yamamoto, M., Araoka, T., Li, Z., et al. (2016). In vivo genome editing via CRISPR/Cas9 mediated homology-independent targeted integration. Nature *540*, 144–149.

Symington, L.S., and Gautier, J. (2011). Double-strand break end resection and repair pathway choice. Annu. Rev. Genet. *45*, 247–271.

Taheri-Ghahfarokhi, A., Taylor, B.J.M., Nitsch, R., Lundin, A., Cavallo, A.L., Madeyski-Bengtson, K., Karlsson, F., Clausen, M., Hicks, R., Mayr, L.M., et al. (2018). Decoding non-random mutational signatures at Cas9 targeted sites. Nucleic Acids Res. *46*, 8417–8434.

Takahashi, I., and Marmur, J. (1963). Replacement of thymidylic acid by deoxyuridylic acid in the deoxyribonucleic acid of a transducing phage for Bacillus subtilis. Nature *197*, 794–795.

Takayama, K., Igai, K., Hagihara, Y., Hashimoto, R., Hanawa, M., Sakuma, T., Tachibana, M., Sakurai, F., Yamamoto, T., and Mizuguchi, H. (2017). Highly efficient biallelic genome editing of human ES/iPS cells using a CRISPR/Cas9 or TALEN system. Nucleic Acids Res. *45*, 5198–5207.

Tang, X., Zhu, Y., Baker, S.L., Bowler, M.W., Chen, B.J., Chen, C., Hogg, J.R., Goff, S.P., and Song, H. (2016). Structural basis of suppression of host translation termination by Moloney Murine Leukemia Virus. Nat. Commun. *7*, 12070.

Teng, F., Cui, T., Feng, G., Guo, L., Xu, K., Gao, Q., Li, T., Li, J., Zhou, Q., and Li, W. (2018). Repurposing CRISPR-Cas12b for mammalian genome engineering. Cell Discov. *4*, 63.

Thompson, P.S., and Cortez, D. (2020). New insights into abasic site repair and tolerance. DNA Repair (Amst.) *90*, 102866.

Tigano, M., Vargas, D.C., Tremblay-Belzile, S., Fu, Y., and Sfeir, A. (2021). Nuclear sensing of breaks in mitochondrial DNA enhances immune surveillance. Nature *591*, 477–481.

Tong, Y., Jørgensen, T.S., Whitford, C.M., Weber, T., and Lee, S.Y. (2021). A versatile genetic engineering toolkit for E. coli based on CRISPR-prime editing. Nat. Commun. *12*, 5206.

Tou, C.J., Schaffer, D.V., and Dueber, J.E. (2020). Targeted Diversification in the *S. cerevisiae* Genome with CRISPR-Guided DNA Polymerase I. ACS Synth. Biol. *9*, 1911–1916.

Truong, L.N., Li, Y., Shi, L.Z., Hwang, P.Y., He, J., Wang, H., Razavian, N., Berns, M.W., and Wu, X. (2013). Microhomology-mediated End Joining and Homologous Recombination share the initial end resection step to repair DNA double-strand breaks in mammalian cells. Proc. Natl. Acad. Sci. USA *110*, 7720–7725.

Urnov, F.D., Miller, J.C., Lee, Y.L., Beausejour, C.M., Rock, J.M., Augustus, S., Jamieson, A.C., Porteus, M.H., Gregory, P.D., and Holmes, M.C. (2005). Highly efficient endogenous human gene correction using designed zinc-finger nucleases. Nature *435*, 646–651.

van Agtmaal, E.L., André, L.M., Willemse, M., Cumming, S.A., van Kessel, I.D.G., van den Broek, W.J.A.A., Gourdon, G., Furling, D., Mouly, V., Monckton, D.G., et al. (2017). CRISPR/Cas9-Induced $(CTG\times CAG)_n$ Repeat Instability in the Myotonic Dystrophy Type 1 Locus: Implications for Therapeutic Genome Editing. Mol. Ther. *25*, 24–43.

van Overbeek, M., Capurso, D., Carter, M.M., Thompson, M.S., Frias, E., Russ, C., Reece-Hoyes, J.S., Nye, C., Gradia, S., Vidal, B., et al. (2016). DNA Repair Profiling Reveals Nonrandom Outcomes at Cas9-Mediated Breaks. Mol. Cell *63*, 633–646.

Velimirovic, M., Zanetti, L.C., Shen, M.W., Fife, J.D., Lin, L., Cha, M., Akinci, E., Barnum, D., Yu, T., and Sherwood, R.I. (2021). Peptide fusion improves prime editing efficiency. bioRxiv. https://doi.org/10.1101/2021.09.22.461415.

Villiger, L., Grisch-Chan, H.M., Lindsay, H., Ringnalda, F., Pogliano, C.B., Allegri, G., Fingerhut, R., Häberle, J., Matos, J., Robinson, M.D., et al. (2018). Treatment of a metabolic liver disease by in vivo genome base editing in adult mice. Nat. Med. *24*, 1519–1525.

Vo, P.L.H., Ronda, C., Klompe, S.E., Chen, E.E., Acree, C., Wang, H.H., and Sternberg, S.H. (2021). CRISPR RNA-guided integrases for high-efficiency, multiplexed bacterial genome engineering. Nat. Biotechnol. *39*, 480–489.

Vriend, L.E., and Krawczyk, P.M. (2017). Nick-initiated homologous recombination: Protecting the genome, one strand at a time. DNA Repair (Amst.) *50*, 1–13.

Walton, R.T., Christie, K.A., Whittaker, M.N., and Kleinstiver, B.P. (2020). Unconstrained genome targeting with near-PAMless engineered CRISPR-Cas9 variants. Science *368*, 290–296.

Wang, Z., and Mosbaugh, D.W. (1989). Uracil-DNA glycosylase inhibitor gene of bacteriophage PBS2 encodes a binding protein specific for uracil-DNA glycosylase. J. Biol. Chem. *264*, 1163–1171.

Wang, L., Xue, W., Yan, L., Li, X., Wei, J., Chen, M., Wu, J., Yang, B., Yang, L., and Chen, J. (2017). Enhanced base editing by co-expression of free uracil DNA glycosylase inhibitor. Cell Res. *27*, 1289–1292.

Wang, X., Li, J., Wang, Y., Yang, B., Wei, J., Wu, J., Wang, R., Huang, X., Chen, J., and Yang, L. (2018). Efficient base editing in methylated regions with a human APOBEC3A-Cas9 fusion. Nat. Biotechnol. *36*, 946–949.

Wang, D., Zhang, F., and Gao, G. (2020a). CRISPR-Based Therapeutic Genome Editing: Strategies and In Vivo Delivery by AAV Vectors. Cell *181*, 136–150.

Wang, S., Zong, Y., Lin, Q., Zhang, H., Chai, Z., Zhang, D., Chen, K., Qiu, J.L., and Gao, C. (2020b). Precise, predictable multi-nucleotide deletions in rice and wheat using APOBEC-Cas9. Nat. Biotechnol. *38*, 1460–1465.

Wang, X., Ding, C., Yu, W., Wang, Y., He, S., Yang, B., Xiong, Y.C., Wei, J., Li, J., Liang, J., et al. (2020c). Cas12a Base Editors Induce Efficient and Specific Editing with Low DNA Damage Response. Cell Rep. *31*, 107723.

Wang, L., Xue, W., Zhang, H., Gao, R., Qiu, H., Wei, J., Zhou, L., Lei, Y.N., Wu, X., Li, X., et al. (2021a). Eliminating base-editor-induced genome-wide and transcriptome-wide off-target mutations. Nat. Cell Biol. *23*, 552–563.

Wang, Q., Liu, J., Janssen, J.M., Le Bouteiller, M., Frock, R.L., and Gonçalves, M.A.F.V. (2021b). Precise and broad scope genome editing based on high-specificity Cas9 nickases. Nucleic Acids Res. *49*, 1173–1198.

Wang, Q., Yang, J., Zhong, Z., Vanegas, J.A., Gao, X., and Kolomeisky, A.B. (2021c). A general theoretical framework to design base editors with reduced bystander effects. Nat. Commun. *12*, 6529.

Webber, B.R., Lonetree, C.L., Kluesner, M.G., Johnson, M.J., Pomeroy, E.J., Diers, M.D., Lahr, W.S., Draper, G.M., Slipek, N.J., Smeester, B.A., et al. (2019). Highly efficient multiplex human T cell engineering without double-strand breaks using Cas9 base. Nat. Commun. *10*, 5222.

Weisheit, I., Kroeger, J.A., Malik, R., Klimmt, J., Crusius, D., Dannert, A., Dichgans, M., and Paquet, D. (2020). Detection of Deleterious On-Target Effects after HDR-Mediated CRISPR Editing. Cell Rep. *31*, 107689.

Wienert, B., Nguyen, D.N., Guenther, A., Feng, S.J., Locke, M.N., Wyman, S.K., Shin, J., Kazane, K.R., Gregory, G.L., Carter, M.A.M., et al. (2020). Timed inhibition of CDC7 increases CRISPR-Cas9 mediated templated repair. Nat. Commun. *11*, 2109.

Wilde, J.J., Aida, T., Del Rosario, R.C.H., Kaiser, T., Qi, P., Wienisch, M., Zhang, Q., Colvin, S., and Feng, G. (2021). Efficient embryonic homozygous gene conversion via RAD51-enhanced interhomolog repair. Cell *184*, 3267–3280.e18.

Wolf, J., Gerber, A.P., and Keller, W. (2002). tadA, an essential tRNA-specific adenosine deaminase from Escherichia coli. EMBO J. *21*, 3841–3851.

Xiao, R., Wang, S., Han, R., Li, Z., Gabel, C., Mukherjee, I.A., and Chang, L. (2021). Structural basis of target DNA recognition by CRISPR-Cas12k for RNA-guided DNA transposition. Mol. Cell *81*, 4457–4466.e5.

Xie, N., Gong, H., Suhl, J.A., Chopra, P., Wang, T., and Warren, S.T. (2016). Reactivation of FMR1 by CRISPR/Cas9-Mediated Deletion of the Expanded CGG-Repeat of the Fragile X Chromosome. PLoS ONE *11*, e0165499.

Xu, L., Wang, J., Liu, Y., Xie, L., Su, B., Mou, D., Wang, L., Liu, T., Wang, X., Zhang, B., et al. (2019). CRISPR-Edited Stem Cells in a Patient with HIV and Acute Lymphocytic Leukemia. N. Engl. J. Med. *381*, 1240–1247.

Xu, P., Liu, Z., Liu, Y., Ma, H., Xu, Y., Bao, Y., Zhu, S., Cao, Z., Wu, Z., Zhou, Z., and Wei, W. (2021a). Genome-wide interrogation of gene functions through base editor screens empowered by barcoded sgRNAs. Nat. Biotechnol. *39*, 1403–1413.

Xu, X., Chemparathy, A., Zeng, L., Kempton, H.R., Shang, S., Nakamura, M., and Qi, L.S. (2021b). Engineered miniature CRISPR-Cas system for mammalian genome regulation and editing. Mol. Cell *81*, 4333–4345.e4.

Yan, S., Tu, Z., Liu, Z., Fan, N., Yang, H., Yang, S., Yang, W., Zhao, Y., Ouyang, Z., Lai, C., et al. (2018). A Huntingtin Knockin Pig Model Recapitulates Features of Selective Neurodegeneration in Huntington's Disease. Cell *173*, 989–1002.e13.

Yan, W.X., Hunnewell, P., Alfonse, L.E., Carte, J.M., Keston-Smith, E., Sothiselvam, S., Garrity, A.J., Chong, S., Makarova, K.S., Koonin, E.V., et al. (2019). Functionally diverse type V CRISPR-Cas systems. Science *363*, 88–91.

Yang, D., Scavuzzo, M.A., Chmielowiec, J., Sharp, R., Bajic, A., and Borowiak, M. (2016). Enrichment of G2/M cell cycle phase in human pluripotent stem cells enhances HDR-mediated gene repair with customizable endonucleases. Sci. Rep. *6*, 21264.

Ye, L., Wang, C., Hong, L., Sun, N., Chen, D., Chen, S., and Han, F. (2018). Programmable DNA repair with CRISPRa/i enhanced homology-directed repair efficiency with a single Cas9. Cell Discov. *4*, 46.

Yeh, W.H., Chiang, H., Rees, H.A., Edge, A.S.B., and Liu, D.R. (2018). In vivo base editing of post-mitotic sensory cells. Nat. Commun. *9*, 2184.

Yeh, C.D., Richardson, C.D., and Corn, J.E. (2019). Advances in genome editing through control of DNA repair pathways. Nat. Cell Biol. *21*, 1468–1478.

Yeh, W.H., Shubina-Oleinik, O., Levy, J.M., Pan, B., Newby, G.A., Wornow, M., Burt, R., Chen, J.C., Holt, J.R., and Liu, D.R. (2020). In vivo base editing restores sensory transduction and transiently improves auditory function in a mouse model of recessive deafness. Sci. Transl. Med. *12*, eaay9101.

Ylä-Herttuala, S. (2018). CRISPR/Cas9 and p53: An Odd Couple Requiring Relationship Management. Mol. Ther. *26*, 2711.

Yu, C., Liu, Y., Ma, T., Liu, K., Xu, S., Zhang, Y., Liu, H., La Russa, M., Xie, M., Ding, S., and Qi, L.S. (2015). Small molecules enhance CRISPR genome editing in pluripotent stem cells. Cell Stem Cell *16*, 142–147.

Yuan, J., Ma, Y., Huang, T., Chen, Y., Peng, Y., Li, B., Li, J., Zhang, Y., Song, B., Sun, X., et al. (2018). Genetic Modulation of RNA Splicing with a CRISPR-Guided Cytidine Deaminase. Mol. Cell *72*, 380–394.e7.

Zafra, M.P., Schatoff, E.M., Katti, A., Foronda, M., Breinig, M., Schweitzer, A.Y., Simon, A., Han, T., Goswami, S., Montgomery, E., et al. (2018). Optimized base editors enable efficient editing in cells, organoids and mice. Nat. Biotechnol. *36*, 888–893.

Zahn, K.E., Jensen, R.B., Wood, R.D., and Doublié, S. (2021). Human DNA polymerase θ harbors DNA end-trimming activity critical for DNA repair. Mol. Cell *81*, 1534–1547.e4.

Zelensky, A.N., Schimmel, J., Kool, H., Kanaar, R., and Tijsterman, M. (2017). Inactivation of Pol θ and C-NHEJ eliminates off-target integration of exogenous DNA. Nat. Commun. *8*, 66.

Zetsche, B., Gootenberg, J.S., Abudayyeh, O.O., Slaymaker, I.M., Makarova, K.S., Essletzbichler, P., Volz, S.E., Joung, J., van der Oost, J., Regev, A., et al. (2015). Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell *163*, 759–771.

Zetsche, B., Heidenreich, M., Mohanraju, P., Fedorova, I., Kneppers, J., DeGennaro, E.M., Winblad, N., Choudhury, S.R., Abudayyeh, O.O., Gootenberg, J.S., et al. (2017). Multiplex gene editing by CRISPR-Cpf1 using a single crRNA array. Nat. Biotechnol. *35*, 31–34.

Zhang, Y., Yuan, F., Presnell, S.R., Tian, K., Gao, Y., Tomkinson, A.E., Gu, L., and Li, G.M. (2005). Reconstitution of 5′-directed human mismatch repair in a purified system. Cell *122*, 693–705.

Zhang, J.P., Li, X.L., Li, G.H., Chen, W., Arakaki, C., Botimer, G.D., Baylink, D., Zhang, L., Wen, W., Fu, Y.W., et al. (2017). Efficient precise knockin with a double cut HDR donor after CRISPR/Cas9-mediated double-stranded DNA cleavage. Genome Biol. *18*, 35.

Zhang, X., Chen, L., Zhu, B., Wang, L., Chen, C., Hong, M., Huang, Y., Li, H., Han, H., Cai, B., et al. (2020a). Increasing the efficiency and targeting range of cytidine base editors through fusion of a single-stranded DNA-binding protein domain. Nat. Cell Biol. *22*, 740–750.

Zhang, X., Zhu, B., Chen, L., Xie, L., Yu, W., Wang, Y., Li, L., Yin, S., Yang, L., Hu, H., et al. (2020b). Dual base editor catalyzes both cytosine and adenine base conversions in human cells. Nat. Biotechnol. *38*, 856–860.

Zhang, Y., Davis, L., and Maizels, N. (2021). Pathways and signatures of mutagenesis at targeted DNA nicks. PLoS Genet. *17*, e1009329.

Zhao, B., Watanabe, G., Morten, M.J., Reid, D.A., Rothenberg, E., and Lieber, M.R. (2019). The essential elements for the noncovalent association of two DNA ends during NHEJ synapsis. Nat. Commun. *10*, 3588.

Zhao, Z., Zhang, H., Xiong, T., Wang, J., Yang, D., Zhu, D., Li, J., Yang, Y., Sun, C., Zhao, Y., and Xi, J.J. (2020). Suppression of *SHROOM1* Improves In Vitro and In Vivo Gene Integration by Promoting Homology-Directed Repair. Int. J. Mol. Sci. *21*, 5821.

Zhao, B., Chen, S.-A.A., Lee, J., and Fraser, H.B. (2021a). Bacterial retrons enable precise gene editing in human cells. bioRxiv. https://doi.org/10.1101/2021.03.29.437260.

Zhao, D., Li, J., Li, S., Xin, X., Hu, M., Price, M.A., Rosser, S.J., Bi, C., and Zhang, X. (2021b). Glycosylase base editors enable C-to-A and C-to-G base changes. Nat. Biotechnol. *39*, 35–40.

Zheng, L., Meng, Y., Campbell, J.L., and Shen, B. (2020). Multiple roles of DNA2 nuclease/helicase in DNA metabolism, genome stability and human diseases. Nucleic Acids Res. *48*, 16–35.

Zheng, C., Liang, S.-Q., Liu, B., Liu, P., Kwan, S.-Y., Wolfe, S.A., and Xue, W. (2021). Development of a flexible split prime editor using truncated reverse transcriptase. bioRxiv. https://doi.org/10.1101/2021.08.26.457801.

Zhuang, Y., Liu, J., Wu, H., Zhu, Q., Yan, Y., Meng, H., Chen, P.R., and Yi, C. (2022). Increasing the efficiency and precision of prime editing with guide RNA pairs. Nat. Chem. Biol. *18*, 29–37.

Zuccaro, M.V., Xu, J., Mitchell, C., Marin, D., Zimmerman, R., Rana, B., Weinstein, E., King, R.T., Palmerola, K.L., Smith, M.E., et al. (2020). Allele-Specific Chromosome Removal after Cas9 Cleavage in Human Embryos. Cell *183*, 1650–1664.e15.

Zuo, E., Huo, X., Yao, X., Hu, X., Sun, Y., Yin, J., He, B., Wang, X., Shi, L., Ping, J., et al. (2017). CRISPR/Cas9-mediated targeted chromosome elimination. Genome Biol. *18*, 224.