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Targeted Gene Therapies: Tools, Applications, Optimization

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Abstract

Many devastating human diseases are caused by mutations in a single gene that prevent a somatic cell from carrying out its essential functions, or by genetic changes acquired as a result of infectious disease or in the course of cell transformation. Targeted gene therapies have emerged as potential strategies for treatment of such diseases. These therapies depend upon rare-cutting endonucleases to cleave at specific sites in or near disease genes. Targeted gene correction provides a template for homology-directed repair, enabling the cell's own repair pathways to erase the mutation and replace it with the correct sequence. Targeted gene disruption ablates the disease gene, disabling its function. Gene targeting can also promote other kinds of genome engineering, including mutation, insertion, or gene deletion. Targeted gene therapies present significant advantages compared to approaches to gene therapy that depend upon delivery of stably expressing transgenes. Recent progress has been fueled by advances in nuclease discovery and design, and by new strategies that maximize efficiency of targeting and minimize off-target damage. Future progress will build on deeper mechanistic understanding of critical factors and pathways.

Keywords

homing endonuclease; zinc finger nuclease; TALE nuclease; homologous recombination; nonhomologous end joining

THE NEED FOR GENE THERAPIES

Gene therapies are emerging as a technically sophisticated but practical approach to treatment of human disease. The term gene therapy is broadly used to describe modifications of the genome that restore function of a defective essential gene or abolish function of a disease gene. The initial strategy was to provide cells with a functional version of a defective gene, much like transgenic mice were generated to carry genes of interest. More recently, targeted strategies for gene therapy have been developed that enlist a cell's own repair pathways to correct or disrupt a target gene.

A wide spectrum of diseases are candidates for treatment by targeted gene therapies. Many devastating human diseases are monogenic disorders, caused by mutations in a single gene. Such genetic deficiencies can now be treated, in principle and increasingly in practice, by correcting the mutation that causes disease. Acquired diseases, including cancer and some infectious diseases, are also candidates for targeted gene therapies. The deregulated cell

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proliferation typical of cancer cells is often the result of mutations that increase expression or activity of oncogenes, and the course of disease could be slowed or arrested by therapies that inactivate those oncogenes. Some infectious diseases — most notably AIDS — are caused by pathogenic microorganisms that take up residence in the nucleus. These and other acquired diseases could be treated by gene disruptions designed to inactivate the infectious agent, or protect cells from infectivity.

TARGETED GENE CORRECTION OR DISRUPTION THERAPIES

Targeted gene therapies enlist a cell's own DNA repair pathways to correct or disrupt a disease gene. The first step is cleavage of the chromosome at a site very near the mutation using rare-cutting endonucleases that recognize long (15-30 bp) DNA sequence motifs predicted to be unique in the targeted genome (Figure 1). Cleavage at the recognition site may then initiate homologous recombination (HR) driven by donor templates that are supplied along with the targeting endonuclease, to effect targeted gene correction. Alternatively, cellular factors associated with the nonhomologous end joining (NHEJ) pathway may create short deletions or insertions that inactivate gene function, to effect targeted gene disruption.

To initiate targeted gene correction, a cell is provided with two essential tools: a nuclease that creates a site-directed DNA lesion, and a DNA template for gene correction. The lesion — either a double-strand break or single-strand break — engages the cellular DNA repair machinery, which then carries out HR with the template DNA (Figure 1, left). This alters the chromosomal DNA sequence to correct the mutation in the target gene *in situ*. Targeted gene correction presents considerable advantages over methods of gene therapy based on delivery of a transgene that supplies an intact copy of the gene. In targeted gene correction, the corrected gene remains in its natural location, under control of its own promoter and enhancer, and no exogenous gene needs to integrate into the genome, diminishing the danger of insertional mutagenesis.

In most eukaryotic cells, the spontaneous frequency of HR at any given locus is low, but can be increased by DNA cleavage. The ability of DNA cleavage to induce HR was first demonstrated in experiments using the homing endonuclease (HE) I-SceI, in yeast (Nickoloff *et al.*, 1986; Plessis *et al.*, 1992), then plant cells (Puchta *et al.*, 1993), and subsequently mammalian cells (Rouet *et al.*, 1994b). This pioneering research led the way to detailed molecular analysis of the mechanisms of HR and NHEJ, central to current targeted gene therapies. Proof of principle that site-directed DNA cleavage was applicable to correcting specific genes was provided by experiments that demonstrated the ability of zinc finger nucleases (ZFNs) to elevate HR frequency in frog oocytes (Bibikova *et al.*, 2001), Drosophila (Bibikova *et al.*, 2003) and human cells (Porteus and Baltimore, 2003).

Like targeted gene correction, targeted gene disruption also depends upon nuclease cleavage. However, it is carried out in the absence of an exogenous donor template using the NHEJ repair pathway, and generally results in short deletions (Figure 1, right). The potential for repair of a targeted DSB to create small deletions was first evident upon analyses of lesions created by I-SceI (Lukacsovich *et al.*, 1994). This observation opened the way to targeted gene disruptions, which are now being applied in therapeutic contexts. For example, the *CCR5* gene encodes a co-receptor essential for HIV-1 infection of T lymphocytes. Targeted disruption has been used to eliminate CCR5 expression, thereby rendering cells resistant to infection by HIV-1 (see Cannon and June, 2011). This application of gene therapy by gene disruption is now in a phase I/II clinical trial (http://clinicaltrials.gov/ct2/show/NCT01252641). Other applications in infectious disease include targeting the latent viral genome to prevent viral replication (Aubert *et al.*, 2011).

Gene disruption could also be used to treat dominant monogenic diseases, such as collagen deficiencies (Chamberlain *et al.*, 2004) or keratin genodermatoses (Bowden, 2011).

TRANSGENIC APPROACHES TO HUMAN GENE THERAPY

Targeted gene therapies contrast with approaches that seek to overcome a deficiency by delivery to the cell of a transgene that encodes a functional gene product. This is sometimes referred to as "gene replacement therapy", even though the defective gene is not in fact replaced but its function complemented by a gene that expresses the missing gene product. This approach to gene therapy has been validated in a number of clinical trials, but it has clear limitations.

One of the earliest genetic disease treated by delivery of a transgene was the severe combined immunodeficiency (SCID) caused by deficiency in adenosine deaminase (ADA). ADA-deficiency affects fewer than 1 in 10^5 live births worldwide, but it is the second most common cause of SCID (Buckley *et al.*, 1997). ADA functions in purine metabolism. In its absence dATP accumulates, inhibiting ribonucleotide reductase, diminishing the dNTP pool and impairing cell proliferation. This has particularly severe consequences for lymphocytes, and causes "combined" immunodeficiency affecting both the B cell and T cell compartments.

ADA-deficiency can be treated by enzyme replacement therapy (i.e, providing the missing enzyme by infusion), but this does not fully restore immune function. To provide a functional gene, retroviral vectors were engineered to transduce the *ADA* gene into T cells (Blaese *et al.*, 1995). Three studies of ADA gene therapy, including over 30 participants, have proven the effectiveness of the treatment (Fischer *et al.*, 2011; Gaspar *et al.*, 2011). This represents a significant advance, despite the fact that the numbers of patients treated is of necessity small as the condition is rare.

The most common severe combined immunodeficiency, SCID-XI, is caused by deficiency in the common γ chain shared by six different cytokine receptors, and encoded by the *IL2RG* gene on the X chromosome. SCID-XI can be treated by hematopoietic stem cell transplant, but appropriate donors cannot always be found. SCID-XI has been successfully treated by gene therapy with retroviral vectors (Hacein-Bey-Abina *et al.*, 2010). Of the first 10 patients involved in the retroviral gene therapy trial, all but one were successfully treated for the genetic deficiency. Unfortunately, four of 10 patients developed T cell leukemia due to insertion of the retrovirus at the *LMO2* proto-oncogene. The leukemia was effectively treated in three of those four patients by chemotherapy, but one of the patients succumbed to the malignancy (Hacein-Bey-Abina *et al.*, 2008).

RARE-CUTTING ENDONUCLEASES, NATURAL AND ENGINEERED

Targeted gene therapies have emerged as potentially safer alternatives to therapies that require delivery of transgenes. There has been a recent explosion of progress in targeted gene therapies fueled largely by development of endonucleases that recognize and cleave specific sites with considerable specificity and efficiency. Three classes of rare-cutting endonucleases are currently used in gene targeting: the naturally occurring Homing Endonucleases and two classes of engineered chimeric proteins, the Zinc Finger Nucleases, and the TAL Effector Nucleases (Figure 2). The long target site recognized by theses enzymes (15-30 bp) provide considerable sequence specificity to DNA cleavage, even in larger genomes. The haploid human genome is 3×10^9 bp in length, so a nuclease that recognizes a 16 bp sequence is predicted in principle to cleave approximately one site, on average ($4^{16} = 4.3 \times 10^9$). In practice, however, rare-cutting endonucleases are not truly sequence-specific. Significant off-target activity has been documented, and one of the

continuing challenges has been to minimize harmful effects associated with rare-cutting nucleases.

Homing Endonucleases

Homing endonucleases (HEs) are naturally occurring sequence-specific endonucleases that recognize and cleave long sequence motifs in double-stranded DNA (Figure 2). They are encoded by introns, and promote intron mobilization by cleaving the recipient gene (Belfort and Roberts, 1997; Guhan and Muniyappa, 2003). HEs were first shown to activate HR in experiments that employed I-SceI cleavage at a reporter site to monitor the outcomes of DNA DSB repair (Rouet *et al.*, 1994a). They have since emerged as promising tools for gene targeting due to their natural ability to recognize long DNA sequence motifs. In this context they are frequently referred to as meganucleases, reflecting their long DNA recognition motifs, not their molecular size. In fact, enzymes in this family tend to be relatively small (30 kD).

To date, hundreds of HEs have been identified in Archaea, Eubacteria and Eukarya and their viruses (Barzel *et al.*, 2011). HEs are classified into five families based on their conserved active site core motif. The best characterized is the LAGLIDADG family. This family includes both homodimeric enzymes such as I-CreI, that target palindromic or near-palindromic consensus motifs, and monomeric enzymes, such as I-SceI and I-AniI, composed of two subdomains that target non-palindromic motifs. Members of the LAGLIDADG family display considerable DNA recognition specificity and have been the main focus of genome engineering applications.

HEs have proven amenable to modification driven by *in silico* analyses. This can be supplemented by *in vitro* directed evolution and selection or high throughput assays to optimize DNA cleavage (Chen and Zhao, 2005; Doyon *et al.*, 2006; Scalley-Kim *et al.*, 2007; Jarjour *et al.*, 2009). Computation-based redesign was first accomplished for I-MsoI (Ashworth *et al.*, 2006). Redesign is still time consuming and predicted variants require considerable experimental validation, so HE therapeutic targets have been chosen in part by virtue of proximity to a promising variant HE recognition motif.

Considerable engineering has focused on the normally dimeric enzyme, I-CreI. Mutations in the *XPC* (Xeroderma pigmentosum C) gene lead to a predisposition to skin cancers due to an inability to repair ultraviolet light-induced DNA damage, and I-CreI has been successfully engineered to generate a heterodimeric derivative that induces efficient targeted recombination in the *XPC* gene (Redondo *et al.*, 2008). I-CreI has also been engineered to produce a monomeric derivative that targets the *RAG-1* gene, which encodes a component of the transposase complex that catalyzes V(D)J recombination in B cells and T cells (Grizot *et al.*, 2009).

Genome mining has enriched the repertoire of HEs and their cognate target sequences. To take advantage of the reservoir of natural diversity, the enormous public sequence databases were searched to create a library containing an estimated 416 unique target ranges for 684 HEs that can be freely accessed at http://homebase-search.tau.ac.il/ (Barzel *et al.*, 2011). Structural conservation permits well-studied HEs to serve as scaffolds onto which specificity determinants identified in such libraries may be grafted, thereby generating novel specificities. For example, grafting of residues predicted to determine target site specificity onto the I-AniI scaffold has created an array of new specificities (Szeto *et al.*, 2011). Engineering of I-OnuI has resulted in a derivative that targets the monoamine oxidase B gene (*MAO-B*), which encodes a component of the mitochondrial outer membrane important in neurotransmitter metabolism and a potential target in neurodegenerative diseases, such as Parkinson disease (Takeuchi *et al.*, 2011). Engineering has also built on a

proprietary database, largely I-CreI-derived, to generate monomeric enzymes that cleave herpes simplex virus to reduce viral load in cultured cells (Grosse *et al.*, 2011).

Modular Engineered Nucleases: ZFNs and TALENs

Two classes of rare-cutting endonuclease, zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), consist of modular DNA-binding domains fused to the nuclease domain of FokI, a type IIS restriction endonuclease (Figure 2). The modularity of these enzymes enables new specificities to be readily engineered by joining DNA recognition domains.

ZFNs—ZFNs (Figure 2) are artificial proteins composed of the DNA binding domain of a zinc finger protein fused to the FokI nuclease domain (Kim *et al.*, 1996; Porteus and Carroll, 2005; Klug, 2010). Specificity is conferred by the zinc finger, a self-contained domain composed of an α-helix which makes contacts with the DNA target, stabilized by two cysteines and two histidines, coordinated by a zinc ion (hence the name). A single zinc finger recognizes a specific 3 bp DNA sequence, and ZFNs can be engineered to recognize longer sequence motifs by fusing individual zinc finger domains. Endonucleolytic activity is the property of the FokI endonuclease domain, which is joined to the C-terminus of the zinc finger modules by a short linker. FokI only cleaves as a dimer, so (unless a recognition motif is palindromic) a heterodimer must be engineered for each DNA half-site, with a FokI monomer linked to each subunit. DNA binding by the zinc fingers then enables FokI dimerization and DNA cleavage in the spacer region between the target DNA sequence motifs.

ZFNs have been used very effectively in genome engineering to enable reverse genetics in a variety of model organisms, as well as in therapeutic contexts (reviewed by Urnov *et al.*, 2011). Utility of ZFNs for gene therapy was established in experiments showing that ZFNs could greatly stimulate reporter gene targeting in human cells (Porteus and Baltimore, 2003). Targeting of a natural chromosomal site, the human *IL2RG* locus, was reported two years later (Urnov *et al.*, 2005). More recently, ZFNs have been applied to genomic modifications of human embryonic stem cells (Hockemeyer *et al.*, 2009; Zou *et al.*, 2009).

TALENs—TALENs (Figure 2) consist of sequence-specific TAL DNA binding domains fused to the FokI nuclease (Bogdanove and Voytas, 2011). The transcription activator-like (TAL) modules derive from plant pathogenic bacteria and normally function to bind specific promoters and activate transcription in the nucleus of the host plant. Some TAL effectors are virulence factors, but the function of most is unknown.

TAL effectors recognize DNA via a central domain, composed of tandem repeats, typically 34 amino acids long. Each repeat binds to one base pair, with specificity determined by two amino acid residues (Boch *et al.*, 2009; Moscou and Bogdanove, 2009). Most natural TAL effectors recognize sequences 15-19 bp in length. The TAL effector domain has been successfully customized to create targeted transcription factors. These have been used to activate genes in plants, their natural hosts (Morbitzer *et al.*, 2011). They have also been adapted to function in human cells, after substitution of a mammalian nuclear localization signal and the transcription activation domain from VP64 (Zhang *et al.*, 2011).

The apparently simple code that TAL effectors use for DNA recognition has spurred interest in developing TAL derivatives that cover the spectrum of specificities desired for genome engineering. The first TALENs were constructed only recently (Christian *et al.*, 2010); nonetheless the readiness with which they can be customized and their efficacy in genome engineering has since been demonstrated in multiple contexts, including gene targeting in human iPS cells (Hockemeyer *et al.*, 2011).

Advantages and applications of the platforms for nuclease-targeted gene therapy

HEs, ZFNs and TALENs are in current use in a great variety of applications, ranging from genomic engineering in model organisms to clinical trials for treatment of human disease. Some of those applications are outlined in Figure 3, and a more detailed list is included in Supplementary Table 1. The list is by no means exhaustive, and the field is currently moving so rapidly that no list can be complete.

The size of the target repertoire is one critical criterion for utility of nucleases used for genome engineering. The repertoire of natural HE sites is relatively small, although genome mining along with *in silico* design and directed evolution has expanded it considerably. Nonetheless, developing a nuclease to cleave near a specific mutation, as is necessary in targeted gene therapies, may prove to be time-consuming and expensive. The modular ZFNs and TALENs have the advantage that they can in principle be designed to cleave nearly any site. TALEN engineering is greatly facilitated by the considerable specificity of DNA binding by TAL modules, especially the ability of individual TAL modules to recognize DNA with neighbor-independent specificity. The recent momentum in customizing TALENs suggests that these may prove to be especially useful in expanding the repertoire of potential targets.

Specificity of cleavage is a second key criterion. None of the targeting nucleases developed thus far exhibits absolute sequence-specificity. As discussed in detail in later sections, improvements in specificity have been achieved by a variety of strategies for nuclease redesign and selection, as well as by improved methods for analysis of off-target cleavage *in vivo*.

Another important criterion for most applications is the ease of delivery to the target cell. When viral vectors are used for nuclease delivery, the size of the gene that encodes the nuclease may become an important consideration. HEs are small (approximately 30 kD), and can be encoded by relatively short genes (approximately 900 bp). ZFNs are somewhat larger, as cleavage requires dimerization of two FokI nuclease domains, each linked to a set of modules designed to recognize one of the two half-sites for binding. Each subunit of a ZFN heterodimer that recognizes an 18 bp recognition sequence is about 30 kD, and the heterodimer is encoded by two genes each approximately 900 bp in length. TALENs are significantly larger. Each 34 residue TAL repeat module recognizes only a single base pair of DNA, and the engineered polypeptide must include approximately 100 residues of additional native TAL sequence N- and C-terminal of the repeats. This means that recognition of 9 bp of DNA requires a monomer of about 500 residues in length, linked to the 200 residue monomeric FokI endonuclease domain for a total of 700 residues. Thus, the predicted size of a functional TALEN dimer is more than 150 kD, encoded by more than 4 kb of DNA. This begins to approach the limits of some viral vectors, although other methods of nuclease delivery, particularly transfection of mRNA (discussed below), appear to offer good alternatives to viral vector-based gene expression.

Alternatives to Nucleases: Using DNA to Target DNA

Several approaches to gene targeting have been reported that rely on the annealing of short oligonucleotides to specify the site of the lesion. Specificity can be determined either by Watson-Crick base pairing or, in the case of triplex forming oligonucleotides (TFOs), by formation of a non-canonical triple helical structure (Mukherjee and Vasquez, 2011). Additionally, the targeting oligonucleotide can itself be used as the donor molecule for gene correction or two oligonucleotides can be used, one to target the lesion and one as donor. DNA-based targeting has been used to correct a mutation in the human dystrophin gene, thus restoring function in a Duchenne muscular dystrophy (DMD) model (Kayali *et al.*,

2010). A similar approach using either TFO or non-TFO oligonucleotides has corrected β-globin mutations (Chin *et al.*, 2008; Lonkar *et al.*, 2009).

Both TFO and non-TFO oligonucleotides can be used for genome modifications alone (reviewed in Aarts and te Riele, 2011) or when conjugated to a DNA damaging agent (de Piedoue *et al.*, 2007; Kim *et al.*, 2007b; Majumdar *et al.*, 2008). An early example of oligonucleotide-directed DNA damage utilized an ^{125}I-TFO to make site-directed DSBs that were repaired *in vitro* by NHEJ (Odersky *et al.*, 2002). Subsequent work has focused on use of psoralen-conjugated oligonucleotides to target interstrand crosslinks (Majumdar *et al.*, 2008; Liu *et al.*, 2009).

CELLULAR PATHWAYS OF DNA BREAK REPAIR

The DNA lesion created by rare-cutting endonucleases can be repaired by distinct repair pathways. HR with an exogenous donor template will yield gene correction, while NHEJ can yield small deletions that disrupt gene function. These pathways both maintain genomic integrity and cell viability in the face of endogenous DNA damage.

Homologous Recombination

HR repairs a DSB by utilizing a homologous DNA template. This aspect of HR renders it “error-free” when the appropriate template is used. HR frequently repairs breaks during the S and G2 phases of the cell cycle when the newly replicated sister chromatid provides an ideal proximal template for DNA repair.

The mechanism of HR has been studied in considerable detail (Heyer *et al.*, 2010). A DSB first undergoes 5’-end resection to leave free 3'-ends (Figure 4, left). The ends become coated with the single-strand DNA (ssDNA) binding protein, RPA; and the strand-annealing protein RAD51 then displaces RPA from the ssDNA, forming a nucleoprotein filament with a free 3’-end that can invade a homologous template. This results in the formation of a D-loop, a critical intermediate. Many accessory factors are required for this step, and for subsequent removal of RAD51 necessary for progression of HR, as discussed in detail elsewhere (Heyer *et al.*, 2010). After D-loop formation, three unique paths are available for repair: Holliday junction (HJ) formation and resolution, break-induced recombination (BIR), and synthesis dependent strand annealing (SDSA), as described below.

Holliday junction formation requires that the second exposed 3’-end also invades the D-loop. This creates an intermediate that can be resolved by cleavage, resulting in physical exchange of genetic material between two DNA duplexes. HR is typically error-free. If it occurs between equivalent positions on sister chromatids, as it normally does in somatic cells, no genetic changes result. However, the identity of the donor for HJ formation is critical. If HR occurs between repetitive sequences, it can result in translocations and other chromosomal rearrangements (Colnaghi *et al.*, 2011). Recombination between homologous regions of two different chromosomes can cause loss of heterozygosity (Blackburn *et al.*, 2004; Larocque *et al.*, 2011). Both loss of heterozygosity and chromosomal rearrangements are potentially oncogenic (O'Keefe *et al.*, 2010; Thompson and Compton, 2010), and this poses one safety concern for targeted gene therapy. Somatic cells have evolved mechanisms that inhibit formation of HJ (Lorenz and Whitby, 2006; Larocque *et al.*, 2011).

BIR can occur when the free 3’-end that created the D-loop primes replication to the end of the chromosome (Llorente *et al.*, 2008). BIR can involve multiple rounds of strand invasion and template switching (Smith *et al.*, 2007). In yeast, BIR results in a high frequency of point mutations (Deem *et al.*, 2011), perhaps due to replication by error-prone polymerases.

SDSA occurs when the free 3' end that created the D-loop primes limited replication, thereby copying genetic information from the template. The newly extended end is then released from the D-loop and anneals with the other end of the processed DSB. SDSA transfers relatively short stretches of sequence and cannot result in chromosomal rearrangements. The cellular mechanisms that inhibit HJ formation in somatic cells may tend to favor SDSA as a pathway for gene correction. One important consideration in targeted gene correction is the length of sequence transferred in the course of repair — the conversion tract — as this determines the necessary proximity of the targeting endonuclease site to the disease-associated mutation. SDSA may be limited in the length of the conversion tract (Taghian and Nickoloff, 1997; Elliott *et al.*, 1998; Larocque and Jasin, 2010), although it may be possible to extend conversion tract length by altering the balance of cellular repair factors.

Nonhomologous End Joining

NHEJ is the predominant pathway of DSB repair in mammalian cells. In NHEJ, two dsDNA ends are ligated together (Figure 4, right). Depending on the nature of the break and the processing that occurs prior to joining, NHEJ can lead to small deletions or insertions. NHEJ can also (though less frequently) join the ends of two distant DSBs resulting in large deletions, inversions and/or chromosomal translocations (reviewed in Mladenov and Iliakis, 2011). The short deletions that accompany NHEJ can be used to promote targeted gene disruption (e.g.; Holt *et al.*, 2010).

NHEJ occurs via two distinct mechanisms; classical NHEJ and alternative, or backup, end joining. Classical NHEJ is a well-characterized pathway that has been thoroughly reviewed (e.g. Mahaney *et al.*, 2009; Mladenov and Iliakis, 2011). In NHEJ, the Ku70-Ku80 heterodimer binds the DNA ends of a DSB where no, or limited, 5'-end resection has occurred. The Ku heterodimer then recruits and activates the DNA-dependent protein kinase catalytic subunit (DNA-PKcs). The XRCC4-LIG4 complex completes the process by ligating the ends together.

Alternative end joining is a distinct pathway from classical NHEJ (Wang *et al.*, 2003; Bennardo *et al.*, 2008). Importantly, alternative end joining has been shown to be responsible for the majority of translocation induced by two DSBs produced concurrently by either I-SceI or ZFNs (Simsek and Jasin, 2010; Simsek *et al.*, 2011) and has been implicated in the formation of oncogenic translocations (reviewed in Nussenzweig and Nussenzweig, 2010). Alternative end joining could, therefore, be a source of chromosomal rearrangements following DNA cleavage, especially if an endonuclease with significant off-target cleavage is used.

Pathway Choice at DSBs: HR vs. NHEJ

The use of the HR and NHEJ pathways is tightly regulated and the "choice" of pathways utilized at a DSB can play a critical role in the success of targeted therapy (Symington and Gautier, 2010). The relative lengths of G1 and S phases of the cell cycle are one determinant, as factors that promote HR are elevated in S phase, and longer G1 phase may favor NHEJ. The structure of the resected DSB is also critical. MRN (MRE11/RAD50/NBS1) is one of the first complexes to bind a DSB. In conjunction with CtIP, MRN can initiate 5'-end resection (Limbo *et al.*, 2007; Sartori *et al.*, 2007). Complexes containing either the EXO1 or DNA2 endonuclease can then further excise, exposing the free 3' end required for HR (Nimonkar *et al.*, 2011). DSBs that do not have a free 3' end will be repaired by NHEJ. Resection, and therefore pathway choice, is regulated by multiple factors including CDK, which may enforce the absence of HR in G1, as well as γH2AX, CtIP, BRCA1 and 53BP1 (e.g.Huertas and Jackson, 2009; Yun and Hiom, 2009; Bouwman *et al.*,

2010; Bunting *et al.*, 2010; Coleman and Greenberg, 2011; Helmink *et al.*, 2011). Most homing endonucleases leave a short (2-5 bp) 3′ overhang at the cut site (Chevalier and Stoddard, 2001), while the FokI endonuclease used in ZFNs and TALENs leaves a short 5' overhang. It remains to be seen whether a difference in polarity of the overhang affects the choice and efficiency of the repair pathway used at a DNA break.

TARGETED GENOME ENGINEERING

Genome engineering in cellular models or model organisms can be carried out by variations on the strategies for gene therapy outlined above, leading to site-directed mutagenesis or precise DNA deletion or transgene insertion (Figure 5). ZFNs have been used to construct a human embryonic stem cell model of Parkinson's disease by introducing specific mutations into the endogenous α-synuclein gene (Soldner *et al.*, 2011). ZFNs have also been used for site-specific insertion of transgenic DNA up to 8kb in length at the human *IL2RG* locus (Moehle *et al.*, 2007), and to tag the endogenous histone H3.3 variants in ES cells, thereby enabling genome wide profiling of this histone mark in different cell types (Goldberg *et al.*, 2010). Targeted genome engineering can also be adapted to generate large DNA deletions, by simultaneous cleavage of a chromosome at two sites to promote deletion of the entire region between the breaks (Figure 5). ZFNs have been used in this sort of application, to create deletions ranging from several hundred to 15 Mbp in human cells (Lee *et al.*, 2010). These are only a few examples of the possibilities offered by rare-cutting nucleases for targeted genome engineering (for a more complete list see Supplementary Table 1).

NUCLEASE AND DONOR DELIVERY

Targeted gene therapies require delivery to the cell of a gene expressing the initiating endonuclease, and therapies dependent upon gene correction also require a donor template. Optimization of delivery of these essential components is an ongoing and active area of research. Viruses evolved to deliver nucleic acids to cells and they are currently vectors of choice for many kinds of gene therapy (Mitchell *et al.*, 2010). Considerable understanding of the advantages and drawbacks of viral vectors emerged from applications of these vectors to transgene delivery. Viruses require specific cell surface receptors for infection, and this natural tropism promotes the use of viral vectors in therapy directed toward some cell types. It is also possible to alter viral packaging to modify natural tropism ("pseudotyping"). However, viral vectors also pose specific drawbacks that are difficult to overcome, including limited cargo capacity, potential for insertional mutagenesis and immunogenicity.

Retrovirus and Lentivirus Vectors

Retroviral vectors have been extensively used to introduce DNA to cells due to their high efficiency of stable delivery and broad cellular tropism, but pose the risk of insertional mutagenesis. Retroviruses are diploid, and a single virion carries two RNA molecules, each 7-10 kb in length. Retroviral vectors for gene delivery need not carry the *gag, pol* and *env* genes which are essential for virion replication, but the genome must include *cis*-regulatory sequences for genome packaging, replication, integration and transgene transcription, which reduces the effective capacity to 5 – 8 kb. Retroviral vectors have been used in applications requiring stable expression of a transgene because integration of a DNA copy of the RNA genome is an essential stage in the retroviral life cycle. Retrovirus integration is not site-specific, but tends to favor regions near promoters, possibly because the open chromatin structure promotes integration. This creates the risk of insertional activation, the most publicized downside to retrovirus vectors. Insertional activation of proto-oncogenes has been documented in independent gene therapy trials treating diseases including X-linked severe combined immunodeficiency (Hacein-Bey-Abina *et al.*, 2008) and chronic

granulomatous disease (Stein *et al.*, 2010). The frequency of these events varies and likely depends on multiple factors including cell type and viral vector.

To minimize the risk of insertional mutagenesis, vector engineering has sought to limit viral integration and activation of adjacent genes by shifting transcriptional activation from the viral LTR promoter to an internal promoter, by separating the promoter from the LTRs with insulator sequences (Shaw and Kohn, 2011), or by directing transgene integration to "safe-harbor" sites (DeKelver *et al.*, 2010; Gaj *et al.*, 2011; Gersbach *et al.*, 2011; Papapetrou *et al.*, 2011). Much of this effort has recently focused on lentiviruses, a genus of retrovirus that establishes infection accompanied by a long latent period. Lentiviruses can infect either dividing or nondividing cells, and establish stable chromosomal integrants. Integration-deficient lentivirus vectors (IDLV), engineered to carry mutations in integrase, exhibit greatly diminished chromosomal insertion, enabling genes or gene products to be supplied only transiently (Yanez-Munoz *et al.*, 2006; Wanisch and Yanez-Munoz, 2009; Matrai *et al.*, 2011). IDLVs have been successfully used for delivery of the nuclease and/or donor template to affect both targeted gene correction and gene disruption (e.g. Cornu and Cathomen, 2007; Lombardo *et al.*, 2007).

Adeno-associated virus vectors (rAAV)

Adeno-associated virus vectors (rAAV) derive from a parvovirus, AAV, with a small single-stranded DNA genome (4.7 kb). AAV requires either a helper virus (e.g. adenovirus, herpes simplex virus) or chemical stimuli to enable efficient replication (Mitchell *et al.*, 2010). Replication proceeds via a circular double-stranded DNA intermediate that can integrate in the chromosome as a single copy or multimers, or form nuclear episomes. There has been recent clinical success in using rAAV to deliver therapeutic genes to treat Leber's congenital amaurosis, a disease of the retina (Maguire *et al.*, 2008) and Parkinson's disease, which affects the central nervous system (Christine *et al.*, 2009). In both retinal and nervous tissue, gene transfer is to immunologically privileged sites comprised of nondividing cells that may favor rAAV episome maintenance. However, rAAV-driven gene therapy of other tissues, such as impaired clotting due to Factor IX deficiency (Manno *et al.*, 2006), has been accompanied by an immune response to viral proteins.

The small size of rAAV precludes delivery of large genes by this vector, such as the gene encoding dystrophin, deficient in Duchenne muscular dystrophy. The small genome also prohibits incorporation of small genes with relatively long regulatory sequences, such as β-globin, a therapeutic target in sickle cell anemia (see Yannaki *et al.*, 2010; Pichavant *et al.*, 2011). Defining minimal sequences that will yield sufficiently regulated expression and still be deliverable by current viral vectors has proven difficult (Yannaki *et al.*, 2010). Some effort has been devoted to the design of minigenes that might provide necessary gene products in trans by encoding only a fraction of the defective host polypeptide. However, such polypeptides may be immunogenic, as evident in experiments that attempted to treat muscular dystrophy with a minigene version of the very large (79 exon) dystrophin gene (Mendell *et al.*, 2010; Moore and Flotte, 2010).

rAAV vectors can also be used to introduce defined sequence changes at homologous chromosomal loci, in the absence of nuclease cleavage (Russell and Hirata, 1998). For example, rAAV was successfully used to inactivate dominant negative mutations in the COL1A1 and COL1A2 collagen genes in mesenchymal cells from individuals with osteogenesis imperfecta (Chamberlain *et al.*, 2004; Chamberlain *et al.*, 2008). However, even though rAAV-mediated gene editing does not require nucleolytic cleavage, efficiency is 100-fold increased by introduction of a DSB in the chromosomal target (Porteus *et al.*, 2003; Metzger *et al.*, 2010).

Vector-free delivery: DNA and mRNA

Delivery of DNA on a vector that cannot replicate has the potential to enhance safety. Plasmids (pDNA) can be delivered to cells in a variety of ways, with the most common being microinjection, electroporation, and lipofection (Gao *et al.*, 2007). The limited lifetime of pDNAs within the nucleus may not only be adequate but also provide a real advantage for genome editing applications, where a limited window of expression of the targeting nuclease may help to minimize off-target cleavage. pDNA exhibit a relatively low frequency of integration, which diminishes but does not abolish the risk of insertional mutagenesis.

Minicircle DNAs are another promising emerging technology. Minicircle DNAs are plasmids that have been stripped of any unnecessary backbone sequences and are generated by a site-specific recombination process. Several studies have already highlighted their superiority in gene delivery, as they are not immunogenic and appear not to be subject to transcriptional silencing by host cells (Chen *et al.*, 2003; Zhao *et al.*, 2010).

mRNA transfection offers another approach that guarantees a brief window of gene expression, with minimal potential for genomic damage. First shown to be effective over 20 years ago (Malone *et al.*, 1989), mRNA transfection has since been very effectively used in immunotherapies that supply autologous cells with antigen receptors that recognize markers on hematopoietic tumor cells (Van Tendeloo *et al.*, 2001; Barrett *et al.*, 2011); and to generate iPS cells by reprogramming diverse cell types (Warren *et al.*, 2010). This may prove to be an effective means for delivery of genes expressing engineered nucleases (particularly TALENs, which are larger than ZFNs or HEs) or factors that modulate repair pathways or chromatin structure, as discussed below. The “hit-and-run” aspect of mRNA transfection makes it especially attractive for delivery of targeting nucleases.

Donor template delivery typically requires the use of vectors, either plasmid or viral, that pose some of the same risks as the vector-borne nuclease delivery mechanisms. The use of short (≤100 nt) single-stranded oligonucleotides as donor templates for nuclease-targeted gene correction could bypass some of these risks. Single-stranded oligonucleotides were initially shown to be effective templates for targeted DSB repair in yeast (Storici *et al.*, 2003). More recently, they have been shown to function efficiently as donor templates for nuclease-targeted gene editing in human cell lines (Radecke *et al.*, 2006; Majumdar *et al.*, 2008; Liu *et al.*, 2010; Chen *et al.*, 2011). One possible concern in a therapeutic context is the potential for sequence insertion between the ends of a targeted DSB (Radecke *et al.*, 2010). The mechanism of transfer of genetic information from a single-stranded oligonucleotide to the chromosome is still undefined, and understanding of mechanism may identify ways to avoid such unwanted outcomes. In any event, this approach will certainly be useful in applications in which the ability to rapidly produce a large number of sequence variants is crucial.

OPTIMIZATION OF TARGETED GENE THERAPIES

Optimization of safety and efficiency of targeted gene therapies is most critical in applications that pertain to human health. In these applications, cells may be scarce and any unintended genetic alteration may have devastating consequences. Optimization of endonuclease activity and specificity should increase efficiency and reduce the danger of off-target breaks. In addition, the modulation of genetic and epigenetic factors should enhance the frequency of the desired outcome, whether correction or disruption. Possible strategies are described below.

Off-Target Cleavage Jeopardizes Safety

DSBs lead to genomic instability, so the potential for off-target cleavage presents an important safety concern for the use of customized endonucleases. Assays of affinity of protein binding to synthetic duplexes *in vitro* provide one measure of nuclease specificity. Such analyses have show that some but not all bases in a DNA binding motif are critical for protein/DNA interaction. However, there is not an absolute correspondence between binding affinity and cleavage (Thyme *et al.*, 2009), so while this analysis is highly suggestive, it does not robustly predict off-target cleavage either *in vitro* or *in vivo*.

Dose-dependent toxicity of ZFNs provided an early alert to the potential of rare-cutter nucleases to carry out off-target cleavage resulting in cell death (Alwin *et al.*, 2005; Porteus, 2006; Cornu *et al.*, 2008; Pruett-Miller *et al.*, 2008). A variety of strategies have been implemented to assay off-target cleavage *in vivo*. These include cytological profiling with established markers of double strand breaks (γ-H2AX, TP53BP1), assessment of rates of random integration, and karyotyping to assay gross chromosomal alterations. By these different measures, expression of ZFNs was found to be associated with considerable off-target cleavage activity (Perez *et al.*, 2008; Gabriel *et al.*, 2011).

Improved methodologies for identification of unintended target sites on a genomewide scale will also contribute to improving the safety of these enzymes. For example, toxicity of I-SceI, an HE, initially appeared relatively low, suggesting that this class of rare-cutter might be more amenable to genomic engineering (Porteus, 2006). However, this was subsequently challenged by experiments that measured genome integration of an adeno-associated virus in I-SceI-treated cells, which revealed a number of noncanonical cleavage sites (Petek *et al.*, 2010). Insertion of integration-deficient lentiviral vectors (IDLVs) has similarly been used to tag sites of ZFN cleavage, thus marking transient and otherwise undetectable DSBs (Gabriel *et al.*, 2011). Specificity has also been tested by coupling systematic identification of target sites *in vitro* with genomewide sequence analyses that assay mutagenesis at cognate cellular sites (Pattanayak *et al.*, 2011).

Improving Target Specificity

ZFNs have been subject to several sorts of refinements to improve specificity and diminish off-target cleavage. The first ZFNs were generated by modular assembly, linking fingers that recognize specific triplet DNA sequences into a multifinger peptide to target specific endogenous loci (Kim *et al.*, 2009). However, many zinc fingers proved to not exhibit strict functional modularity; instead, sequence-specificity was influenced by neighboring fingers, resulting in off-target cleavage.

This necessitated development of alternative strategies to identify ZFNs that recognized a target site with robust specificity. Some strategies involve structure-based redesign of protein/DNA contacts (Alibes *et al.*, 2010). Others rely on bacterial selection systems to identify finger combinations that work well together (Maeder *et al.*, 2008; Pruett-Miller *et al.*, 2008) or improve ZFN specificity (e.g. Guo *et al.*, 2010). Selections may start either from a large random library of ZFNs, or from archived pools pre-selected to recognize specific sequences (Maeder *et al.*, 2008; Kim *et al.*, 2011; Sander *et al.*, 2011; Urnov *et al.*, 2011). These strategies have led to a new generation of ZFNs that exhibit significantly less toxicity than their predecessors (Miller *et al.*, 2007; Szczepek *et al.*, 2007; Handel *et al.*, 2009; Pruett-Miller *et al.*, 2009; Gupta *et al.*, 2011; Ramalingam *et al.*, 2011).

ZFNs and TALENs both use the Fok I catalytic domain for nuclease cleavage. Some off-target effects may be attributed to the mechanistic properties of the Fok I domain (Halford *et al.*, 2011). The Fok I nuclease domain must dimerize to cleave. Enzymes are designed with the intention that the ZFN domains bind DNA half-sites contained on a short contiguous

target, and direct Fok I cleavage specifically to that region. However, interaction of a DNA-bound subunit with a free subunit may stimulate cleavage, making it difficult to avoid off-target cleavage at half-sites. Another source of off-target cleavage is homodimerization, which enables recognition of a palindromic site composed of two half-sites. Modification of the architecture of the dimer interface has been carried out to prevent homodimerization of ZFN subunits (Miller *et al.*, 2007; Szczepek *et al.*, 2007; Sollu *et al.*, 2010; Doyon *et al.*, 2011; Ramalingam *et al.*, 2011).

Although specificity testing of TALENs and HEs has not been as extensive as for ZFNs, these classes of enzymes seem to have clear therapeutic potential with less re-engineering. In gene editing experiments, TALENs performed as well as ZFNs with regard to efficacy and showed greater specificity. In a study targeting endogenous loci in yeast, no evidence of off-target site was found using whole genome sequencing (Li *et al.*, 2011). In human stem cells, only two of 19 potential off target sites were found to be occasionally disrupted but much less frequently than the intended target (Hockemeyer *et al.*, 2011). Perhaps most significantly, in a side-by-side comparison of ZFNs and TALENs targeting the *CCR5* and *IL2RG* genes, both nucleases showed similar gene disruption activities but TALEN showed much reduced cytotoxicity and off-target cleavage at the *CCR2* locus (Mussolino *et al.*, 2011).

Improving Safety: Initiation of Targeted Gene Correction by Nicks, not DSBs

The ability of DNA nicks (single-strand breaks) to initiate homologous recombination was first established in yeasts (Strathern *et al.*, 1991; Arcangioli, 1998). These results were extended to mammalian cells in experiments that showed recombination could be stimuated by derivatives of the RAG1/RAG2 nuclease that can generate nicks, but not DSBs (Lee *et al.*, 2004). More recently, DNA nicks have been shown to initiate targeted gene correction nearly as efficiently as DSBs, and they appear to have much more desirable safety properties. Initial experiments showed that a derivative of the monomeric HE, I-AniI, mutated at one if its two active sites, could promote targeted gene correction of either an episomal or chromosomal reporter (McConnell Smith *et al.*, 2009). Subsequently, use of a reporter that can measure either targeted gene correction or disruption resulting from a DSB or nick at a single site (Certo *et al.*, 2011) permitted direct comparison of the efficiency and safety of these two kinds of DNA lesions. Nicks were shown to initiate targeted gene correction nearly as efficiently as DSBs, but to cause orders of magnitude less NHEJ, a very desirable safety profile for gene correction (Davis and Maizels, 2011).

The HEs, ZFNs and TALENs in current use for targeted gene therapies can be readily converted from enzymes that cut both DNA strands to enzymes that nick DNA. Monomeric HEs can be converted, by a point mutation in one of their two active sites, a conversion first reported for I-SceI (Niu *et al.*, 2008). This should be applicable to most, if not all, monomeric LAGLIDADG HEs. FokI, which provides the endonuclease domain for the modular engineered ZFNs and TALENs, can similarly be redesigned as a nickase (Sanders *et al.*, 2009) that may be incorporated into the current ZFN and TALEN platforms (Maeder *et al.*, 2008; Cermak *et al.*, 2011; Li *et al.*, 2011). Thus, the use of nicks rather than DSBs should be generally applicable for efficient and safe DNA targeting.

Improving Efficiency: Modulation of Repair Factors

Modulation of repair factor levels or activity has clear potential to enhance the efficiency of targeted gene correction. For example, enhanced expression of RAD51 paralogs has been shown to increase not only the frequency of HR but also the length of gene conversion tracts (Nagaraju *et al.*, 2006; Nagaraju *et al.*, 2009; Ordinario *et al.*, 2009). Longer repair tracts could extend the range of an endonuclease engineered to target a specific gene.

siRNA knockdowns also have the potential to stimulate targeted gene correction or enhance its safety. A recent screen of siRNAs targeting more than 19,000 human genes identified 64 genes that affected the frequency of HR between chromosome and exogenous target (Delacote *et al.*, 2011). Even if modulation of levels of a single factor has only a modest (several-fold) effect, judicious combinations may provide sufficiently improved efficiency to enable applications in therapeutic contexts, where initial cell numbers may be limiting. Repair pathway modulation does have the potential to perturb global genome stability (Moynahan and Jasin, 2010), and careful monitoring will be necessary to ensure that gains in efficiency are not compromised by loss of safety.

Given the tremendous potential of stem cells in clinical uses of TGE (Narsinh and Wu, 2010; Rahman *et al.*, 2011), understanding details of DNA repair in these cells is of particular importance. Several recent studies have demonstrated that NHEJ in human embryonic stem cells (hESCs) is predominantly independent of ATM and DNA-PKcs. This is in contrast to NHEJ in hESC-derived neural progenitors and astrocytes (Adams *et al.*, 2010a; Adams *et al.*, 2010b). This alternative regulation of NHEJ could be the basis of elevated levels of NHEJ observed in hESCs (Fan *et al.*, 2011). Consistent with these findings, levels of HR have been observed to be reduced in hESCs cell lines relative to differentiated cell lines (Fung and Weinstock, 2011). Interestingly, hESCs and iPS cells have a relatively large proportion of cells in S phase (Fan *et al.*, 2011; Ghule *et al.*, 2011), the cell cycle phase that favors HR. Perhaps the alternative regulation of DNA repair indicated by the ATM and DNA-PKcs independence of NHEJ is necessitated by a requirement for both rapid proliferation (short G1) and efficient DNA repair (NHEJ).

Modulation of Chromatin Structure

Chromatin structure may affect the choice and efficiency of repair pathway at the DSB. Studies comparing DSB repair efficiency in condensed vs. relaxed chromatin regions showed that DSBs induced in heterochromatic regions have slower repair kinetics than those induced in euchromatic regions (Cowell *et al.*, 2007; Kim *et al.*, 2007a; Goodarzi *et al.*, 2010). The chromosomal genes targeted for correction or disruption are likely to vary in terms of DNA accessibility, so the modulation of chromatin structure holds considerable promise for enhancing the efficiency and possibly safety of targeted gene therapy.

Treatment of cells with histone deacetylase inhibitors can diminish the effects of repressive chromatin but this may be accompanied by side effects that are especially undesirable in therapeutic contexts (Mehnert and Kelly, 2007). An alternative approach is to identify specific factors that may respond to downregulation by specific siRNAs. Supporting this approach, one factor identified and vetted for functionality in a recent genomewide screen for siRNAs that promote targeted gene correction has proven to be involved in chromatin remodeling (Delacote *et al.*, 2011).

It may also be possible to recruit chromatin-associated proteins to a lesion to favor a specific DNA repair pathway. Chromatin-associated proteins are rapidly mobilized to and from DNA breaks and include histone modifying enzymes, chromatin-remodeling factors, and DNA methyltransferase enzymes (Polo and Jackson, 2011). Interestingly, DSB modification of H3K56 occurs in a biphasic manner, initially undergoing rapid deacetylation by histone deacetylases (HDACs) 1 and 2 (Miller *et al.*, 2010) that may promote NHEJ, followed by acetylation to favor HR. It may therefore be possible to modulate histone acetylation to tip the balance toward HR or NHEJ, depending on the application. One specific target in this pathway may be the histone deacetylase SIRT6, which has been shown to accumulate at DSBs induced by HEs, where it is necessary for stabilization of PKcs, a key factor in NHEJ (McCord *et al.*, 2009).

Chromatin modifiers can be targeted to specific genomic sites upon expression as chimeras fused to DNA binding domains of factors that recognize specific sequence motifs (Cummings *et al.*, 2007; Cummings *et al.*, 2008; Soutoglou and Misteli, 2008; Yabuki *et al.*, 2009). This approach is conceptually analogous to fusion of a nuclease domain to a DNA binding domain to engineer a ZFN or TALEN. It offers the potential to maintain the delicate balance of repair necessary for genome integrity while producing the desired outcome at the target site.

Controlling epigenetic modifications of delivery vectors may also enhance targeted gene correction. As discussed above, pDNA and viral vectors have been used to deliver the targeting nuclease and repair template to the target cell. pDNA can assemble into nucleosome-containing minichromosomes (Reeves *et al.*, 1985) and undergo methylation that leads to intracellular silencing (Hong *et al.*, 2001). Similarly, viral vectors, including rAAVs and IDLVs, are chromatinized following cell entry (Okada *et al.*, 2006; Kantor *et al.*, 2009). Therefore, strategies to counter epigenetic silencing will have to be developed, such as the use of insulator sequences (reviewed in Raab and Kamakaka, 2010).

Future perspectives

Targeted gene therapies are still in the early stages of development, but hold significant therapeutic promise. Progress will depend upon developing endonucleases for exquisitely specific cleavage in the context of the entire genome, enhancing the efficiency of HR, and minimizing the potential for DNA damage and translocation. Future advances in all these areas will depend on deeper understanding of the molecular mechanisms that underlie genomic stability and instability in human cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Aarts M, te Riele H. Progress and prospects: oligonucleotide-directed gene modification in mouse embryonic stem cells: a route to therapeutic application. Gene Ther. 2011; 18:213–219. [PubMed: 21160530]

Adams BR, Golding SE, Rao RR, Valerie K. Dynamic dependence on ATR and ATM for double-strand break repair in human embryonic stem cells and neural descendants. PLoS One. 2010a; 5:e10001. [PubMed: 20368801]

Adams BR, Hawkins AJ, Povirk LF, Valerie K. ATM-independent, high-fidelity nonhomologous end joining predominates in human embryonic stem cells. Aging (Albany NY). 2010b; 2:582–596. [PubMed: 20844317]

Alibes A, Serrano L, Nadra AD. Structure-based DNA-binding prediction and design. Methods Mol Biol. 2010; 649:77–88. [PubMed: 20680828]

Alwin S, Gere MB, Guhl E, Effertz K, Barbas CF 3rd, Segal DJ, Weitzman MD, Cathomen T. Custom zinc-finger nucleases for use in human cells. Mol Ther. 2005; 12:610–617. [PubMed: 16039907]

Arcangioli B. A site- and strand-specific DNA break confers asymmetric switching potential in fission yeast. EMBO J. 1998; 17:4503–4510. [PubMed: 9687516]

Ashworth J, Havranek JJ, Duarte CM, Sussman D, Monnat RJ Jr. Stoddard BL, Baker D. Computational redesign of endonuclease DNA binding and cleavage specificity. Nature. 2006; 441:656–659. [PubMed: 16738662]

Aubert M, Ryu BY, Banks L, Rawlings DJ, Scharenberg AM, Jerome KR. Successful targeting and disruption of an integrated reporter lentivirus using the engineered homing endonuclease Y2 I-AniI. PLoS One. 2011; 6:e16825. [PubMed: 21399673]

Barrett DM, Zhao Y, Liu X, Jiang S, Carpenito C, Kalos M, Carroll RG, June CH, Grupp SA. Treatment of advanced leukemia in mice with mRNA engineered T cells. Hum Gene Ther. 2011

Barzel A, Privman E, Peeri M, Naor A, Shachar E, Burstein D, Lazary R, Gophna U, Pupko T, Kupiec M. Native homing endonucleases can target conserved genes in humans and in animal models. Nucleic Acids Res. 2011; 39:6646–6659. [PubMed: 21525128]

Belfort M, Roberts RJ. Homing endonucleases: keeping the house in order. Nucleic Acids Res. 1997; 25:3379–3388. [PubMed: 9254693]

Bennardo N, Cheng A, Huang N, Stark JM. Alternative-NHEJ is a mechanistically distinct pathway of mammalian chromosome break repair. PLoS Genet. 2008; 4:e1000110. [PubMed: 18584027]

Bibikova M, Beumer K, Trautman JK, Carroll D. Enhancing gene targeting with designed zinc finger nucleases. Science (New York, NY). 2003; 300:764.

Bibikova M, Carroll D, Segal DJ, Trautman JK, Smith J, Kim YG, Chandrasegaran S. Stimulation of homologous recombination through targeted cleavage by chimeric nucleases. Mol Cell Biol. 2001; 21:289–297. [PubMed: 11113203]

Blackburn AC, McLary SC, Naeem R, Luszcz J, Stockton DW, Donehower LA, Mohammed M, Mailhes JB, Soferr T, Naber SP, Otis CN, Jerry DJ. Loss of heterozygosity occurs via mitotic recombination in Trp53+/- mice and associates with mammary tumor susceptibility of the BALB/c strain. Cancer Res. 2004; 64:5140–5147. [PubMed: 15289317]

Blaese RM, Culver KW, Miller AD, Carter CS, Fleisher T, Clerici M, Shearer G, Chang L, Chiang Y, Tolstoshev P, Greenblatt JJ, Rosenberg SA, Klein H, Berger M, Mullen CA, Ramsey WJ, Muul L, Morgan RA, Anderson WF. T lymphocyte-directed gene therapy for ADA- SCID: initial trial results after 4 years. Science. 1995; 270:475–480. [PubMed: 7570001]

Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, Lahaye T, Nickstadt A, Bonas U. Breaking the code of DNA binding specificity of TAL-type III effectors. Science. 2009; 326:1509–1512. [PubMed: 19933107]

Bogdanove AJ, Voytas DF. TAL effectors: customizable proteins for DNA targeting. Science. 2011; 333:1843–1846. [PubMed: 21960622]

Bouwman P, Aly A, Escandell JM, Pieterse M, Bartkova J, van der Gulden H, Hiddingh S, Thanasoula M, Kulkarni A, Yang Q, Haffty BG, Tommiska J, Blomqvist C, Drapkin R, Adams DJ, Nevanlinna H, Bartek J, Tarsounas M, Ganesan S, Jonkers J. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. Nat Struct Mol Biol. 2010; 17:688–695. [PubMed: 20453858]

Bowden PE. Gene therapy for keratin genodermatoses: striving forward but obstacles persist. J Invest Dermatol. 2011; 131:1403–1405. [PubMed: 21673708]

Buckley RH, Schiff RI, Schiff SE, Markert ML, Williams LW, Harville TO, Roberts JL, Puck JM. Human severe combined immunodeficiency: genetic, phenotypic, and functional diversity in one hundred eight infants. J Pediatr. 1997; 130:378–387. [PubMed: 9063412]

Bunting SF, Callen E, Wong N, Chen HT, Polato F, Gunn A, Bothmer A, Feldhahn N, Fernandez-Capetillo O, Cao L, Xu X, Deng CX, Finkel T, Nussenzweig M, Stark JM, Nussenzweig A. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell. 2010; 141:243–254. [PubMed: 20362325]

Cannon P, June C. Chemokine receptor 5 knockout strategies. Curr Opin HIV AIDS. 2011; 6:74–79. [PubMed: 21242897]

Cermak T, Doyle EL, Christian M, Wang L, Zhang Y, Schmidt C, Baller JA, Somia NV, Bogdanove AJ, Voytas DF. Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. Nucleic Acids Res. 2011; 39:e82. [PubMed: 21493687]

Certo MT, Ryu BY, Annis JE, Garibov M, Jarjour J, Rawlings DJ, Scharenberg AM. Tracking genome engineering outcome at individual DNA breakpoints. Nat Methods. 2011; 8:671–676. [PubMed: 21743461]

Chamberlain JR, Deyle DR, Schwarze U, Wang P, Hirata RK, Li Y, Byers PH, Russell DW. Gene targeting of mutant COL1A2 alleles in mesenchymal stem cells from individuals with osteogenesis imperfecta. Mol Ther. 2008; 16:187–193. [PubMed: 17955022]

Chamberlain JR, Schwarze U, Wang PR, Hirata RK, Hankenson KD, Pace JM, Underwood RA, Song KM, Sussman M, Byers PH, Russell DW. Gene targeting in stem cells from individuals with osteogenesis imperfecta. Science. 2004; 303:1198–1201. [PubMed: 14976317]

Chen F, Pruett-Miller SM, Huang Y, Gjoka M, Duda K, Taunton J, Collingwood TN, Frodin M, Davis GD. High-frequency genome editing using ssDNA oligonucleotides with zinc-finger nucleases. Nat Methods. 2011; 8:753–755. [PubMed: 21765410]

Chen Z, Zhao H. A highly sensitive selection method for directed evolution of homing endonucleases. Nucleic Acids Res. 2005; 33:e154. [PubMed: 16214805]

Chen ZY, He CY, Ehrhardt A, Kay MA. Minicircle DNA vectors devoid of bacterial DNA result in persistent and high-level transgene expression in vivo. Mol Ther. 2003; 8:495–500. [PubMed: 12946323]

Chevalier BS, Stoddard BL. Homing endonucleases: structural and functional insight into the catalysts of intron/intein mobility. Nucleic Acids Res. 2001; 29:3757–3774. [PubMed: 11557808]

Chin JY, Kuan JY, Lonkar PS, Krause DS, Seidman MM, Peterson KR, Nielsen PE, Kole R, Glazer PM. Correction of a splice-site mutation in the beta-globin gene stimulated by triplex-forming peptide nucleic acids. Proc Natl Acad Sci U S A. 2008; 105:13514–13519. [PubMed: 18757759]

Christian M, Cermak T, Doyle EL, Schmidt C, Zhang F, Hummel A, Bogdanove AJ, Voytas DF. Targeting DNA double-strand breaks with TAL effector nucleases. Genetics. 2010; 186:757–761. [PubMed: 20660643]

Christine CW, Starr PA, Larson PS, Eberling JL, Jagust WJ, Hawkins RA, VanBrocklin HF, Wright JF, Bankiewicz KS, Aminoff MJ. Safety and tolerability of putaminal AADC gene therapy for Parkinson disease. Neurology. 2009; 73:1662–1669. [PubMed: 19828868]

Coleman KA, Greenberg RA. The BRCA1-RAP80 complex regulates DNA repair mechanism utilization by restricting end resection. J Biol Chem. 2011; 286:13669–13680. [PubMed: 21335604]

Colnaghi R, Carpenter G, Volker M, O'Driscoll M. The consequences of structural genomic alterations in humans: Genomic Disorders, genomic instability and cancer. Semin Cell Dev Biol. 2011

Cornu TI, Cathomen T. Targeted genome modifications using integrase-deficient lentiviral vectors. Mol Ther. 2007; 15:2107–2113. [PubMed: 17998901]

Cornu TI, Thibodeau-Beganny S, Guhl E, Alwin S, Eichtinger M, Joung JK, Cathomen T. DNA-binding specificity is a major determinant of the activity and toxicity of zinc-finger nucleases. Mol Ther. 2008; 16:352–358. [PubMed: 18026168]

Cowell IG, Sunter NJ, Singh PB, Austin CA, Durkacz BW, Tilby MJ. gammaH2AX foci form preferentially in euchromatin after ionising-radiation. PLoS One. 2007; 2:e1057. [PubMed: 17957241]

Cummings WJ, Bednarski DW, Maizels N. Genetic variation stimulated by epigenetic modification. PLoS One. 2008; 3:e4075. [PubMed: 19115012]

Cummings WJ, Yabuki M, Ordinario EC, Bednarski DW, Quay S, Maizels N. Chromatin structure regulates gene conversion. PLoS Biol. 2007; 5:e246. [PubMed: 17880262]

Davis L, Maizels N. DNA nicks promote efficient and safe targeted gene correction. PLoS One. 2011; 6:e23981. [PubMed: 21912657]

de Piedoue G, Andrieu-Soler C, Concordet JP, Maurisse R, Sun JS, Lopez B, Kuzniak I, Leboulch P, Feugeas JP. Targeted gene correction with 5' acridine-oligonucleotide conjugates. Oligonucleotides. 2007; 17:258–263. [PubMed: 17638529]

Deem A, Keszthelyi A, Blackgrove T, Vayl A, Coffey B, Mathur R, Chabes A, Malkova A. Break-induced replication is highly inaccurate. PLoS Biol. 2011; 9:e1000594. [PubMed: 21347245]

DeKelver RC, Choi VM, Moehle EA, Paschon DE, Hockemeyer D, Meijsing SH, Sancak Y, Cui X, Steine EJ, Miller JC, Tam P, Bartsevich VV, Meng X, Rupniewski I, Gopalan SM, Sun HC, Pitz KJ, Rock JM, Zhang L, Davis GD, Rebar EJ, Cheeseman IM, Yamamoto KR, Sabatini DM, Jaenisch R, Gregory PD, Urnov FD. Functional genomics, proteomics, and regulatory DNA

analysis in isogenic settings using zinc finger nuclease-driven transgenesis into a safe harbor locus in the human genome. Genome research. 2010; 20:1133–1142. [PubMed: 20508142]

Delacote F, Perez C, Guyot V, Mikonio C, Potrel P, Cabaniols JP, Delenda C, Paques F, Duchateau P. Identification of genes regulating gene targeting by a high-throughput screening approach. J Nucleic Acids. 2011; 2011:947212. [PubMed: 21716659]

Doyon JB, Pattanayak V, Meyer CB, Liu DR. Directed evolution and substrate specificity profile of homing endonuclease I-SceI. J Am Chem Soc. 2006; 128:2477–2484. [PubMed: 16478204]

Doyon Y, Vo TD, Mendel MC, Greenberg SG, Wang J, Xia DF, Miller JC, Urnov FD, Gregory PD, Holmes MC. Enhancing zinc-finger-nuclease activity with improved obligate heterodimeric architectures. Nat Methods. 2011; 8:74–79. [PubMed: 21131970]

Elliott B, Richardson C, Winderbaum J, Nickoloff JA, Jasin M. Gene conversion tracts from double-strand break repair in mammalian cells. Mol Cell Biol. 1998; 18:93–101. [PubMed: 9418857]

Fan J, Robert C, Jang YY, Liu H, Sharkis S, Baylin SB, Rassool FV. Human induced pluripotent cells resemble embryonic stem cells demonstrating enhanced levels of DNA repair and efficacy of nonhomologous end-joining. Mutat Res. 2011; 713:8–17. [PubMed: 21718709]

Fischer A, Hacein-Bey-Abina S, Cavazzana-Calvo M. Gene therapy for primary adaptive immune deficiencies. J Allergy Clin Immunol. 2011; 127:1356–1359. [PubMed: 21624615]

Fung H, Weinstock DM. Repair at single targeted DNA double-strand breaks in pluripotent and differentiated human cells. PLoS One. 2011; 6:e20514. [PubMed: 21633706]

Gabriel R, Lombardo A, Arens A, Miller JC, Genovese P, Kaeppel C, Nowrouzi A, Bartholomae CC, Wang J, Friedman G, Holmes MC, Gregory PD, Glimm H, Schmidt M, Naldini L, von Kalle C. An unbiased genome-wide analysis of zinc-finger nuclease specificity. Nat Biotechnol. 2011

Gaj T, Mercer AC, Gersbach CA, Gordley RM, Barbas CF 3rd. Structure-guided reprogramming of serine recombinase DNA sequence specificity. Proc Natl Acad Sci U S A. 2011; 108:498–503. [PubMed: 21187418]

Gao X, Kim KS, Liu D. Nonviral gene delivery: what we know and what is next. AAPS J. 2007; 9:E92–104. [PubMed: 17408239]

Gaspar HB, Cooray S, Gilmour KC, Parsley KL, Zhang F, Adams S, Bjorkegren E, Bayford J, Brown L, Davies EG, Veys P, Fairbanks L, Bordon V, Petropolou T, Kinnon C, Thrasher AJ. Hematopoietic stem cell gene therapy for adenosine deaminase-deficient severe combined immunodeficiency leads to long-term immunological recovery and metabolic correction. Sci Transl Med. 2011; 3:97ra80.

Gersbach CA, Gaj T, Gordley RM, Mercer AC, Barbas CF 3rd. Targeted plasmid integration into the human genome by an engineered zinc-finger recombinase. Nucleic Acids Res. 2011; 39:7868–7878. [PubMed: 21653554]

Ghule PN, Medina R, Lengner CJ, Mandeville M, Qiao M, Dominski Z, Lian JB, Stein JL, van Wijnen AJ, Stein GS. Reprogramming the pluripotent cell cycle: restoration of an abbreviated G1 phase in human induced pluripotent stem (iPS) cells. J Cell Physiol. 2011; 226:1149–1156. [PubMed: 20945438]

Goldberg AD, Banaszynski LA, Noh KM, Lewis PW, Elsaesser SJ, Stadler S, Dewell S, Law M, Guo X, Li X, Wen D, Chapgier A, DeKelver RC, Miller JC, Lee YL, Boydston EA, Holmes MC, Gregory PD, Greally JM, Rafii S, Yang C, Scambler PJ, Garrick D, Gibbons RJ, Higgs DR, Cristea IM, Urnov FD, Zheng D, Allis CD. Distinct factors control histone variant H3.3 localization at specific genomic regions. Cell. 2010; 140:678–691. [PubMed: 20211137]

Goodarzi AA, Jeggo P, Lobrich M. The influence of heterochromatin on DNA double strand break repair: Getting the strong, silent type to relax. DNA Repair (Amst). 2010; 9:1273–1282. [PubMed: 21036673]

Grizot S, Smith J, Daboussi F, Prieto J, Redondo P, Merino N, Villate M, Thomas S, Lemaire L, Montoya G, Blanco FJ, Paques F, Duchateau P. Efficient targeting of a SCID gene by an engineered single-chain homing endonuclease. Nucleic Acids Res. 2009; 37:5405–5419. [PubMed: 19584299]

Grosse S, Huot N, Mahiet C, Arnould S, Barradeau S, Clerre DL, Chion-Sotinel I, Jacqmarcq C, Chapellier B, Ergani A, Desseaux C, Cedrone F, Conseiller E, Paques F, Labetoulle M, Smith J.

Meganuclease-mediated Inhibition of HSV1 Infection in Cultured Cells. Mol Ther. 2011; 19:694–702. [PubMed: 21224832]

Guhan N, Muniyappa K. Structural and functional characteristics of homing endonucleases. Crit Rev Biochem Mol Biol. 2003; 38:199–248. [PubMed: 12870715]

Guo J, Gaj T, Barbas CF 3rd. Directed evolution of an enhanced and highly efficient FokI cleavage domain for zinc finger nucleases. J Mol Biol. 2010; 400:96–107. [PubMed: 20447404]

Gupta A, Meng X, Zhu LJ, Lawson ND, Wolfe SA. Zinc finger protein-dependent and -independent contributions to the in vivo off-target activity of zinc finger nucleases. Nucleic Acids Res. 2011; 39:381–392. [PubMed: 20843781]

Hacein-Bey-Abina S, Garrigue A, Wang GP, Soulier J, Lim A, Morillon E, Clappier E, Caccavelli L, Delabesse E, Beldjord K, Asnafi V, MacIntyre E, Dal Cortivo L, Radford I, Brousse N, Sigaux F, Moshous D, Hauer J, Borkhardt A, Belohradsky BH, Wintergerst U, Velez MC, Leiva L, Sorensen R, Wulffraat N, Blanche S, Bushman FD, Fischer A, Cavazzana-Calvo M. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. J Clin Invest. 2008; 118:3132–3142. [PubMed: 18688285]

Hacein-Bey-Abina S, Hauer J, Lim A, Picard C, Wang GP, Berry CC, Martinache C, Rieux-Laucat F, Latour S, Belohradsky BH, Leiva L, Sorensen R, Debre M, Casanova JL, Blanche S, Durandy A, Bushman FD, Fischer A, Cavazzana-Calvo M. Efficacy of gene therapy for X-linked severe combined immunodeficiency. N Engl J Med. 2010; 363:355–364. [PubMed: 20660403]

Halford SE, Catto LE, Pernstich C, Rusling DA, Sanders KL. The reaction mechanism of FokI excludes the possibility of targeting zinc finger nucleases to unique DNA sites. Biochem Soc Trans. 2011; 39:584–588. [PubMed: 21428944]

Handel EM, Alwin S, Cathomen T. Expanding or restricting the target site repertoire of zinc-finger nucleases: the inter-domain linker as a major determinant of target site selectivity. Mol Ther. 2009; 17:104–111. [PubMed: 19002164]

Helmink BA, Tubbs AT, Dorsett Y, Bednarski JJ, Walker LM, Feng Z, Sharma GG, McKinnon PJ, Zhang J, Bassing CH, Sleckman BP. H2AX prevents CtIP-mediated DNA end resection and aberrant repair in G1-phase lymphocytes. Nature. 2011; 469:245–249. [PubMed: 21160476]

Heyer WD, Ehmsen KT, Liu J. Regulation of homologous recombination in eukaryotes. Annu Rev Genet. 2010; 44:113–139. [PubMed: 20690856]

Hockemeyer D, Soldner F, Beard C, Gao Q, Mitalipova M, DeKelver RC, Katibah GE, Amora R, Boydston EA, Zeitler B, Meng X, Miller JC, Zhang L, Rebar EJ, Gregory PD, Urnov FD, Jaenisch R. Efficient targeting of expressed and silent genes in human ESCs and iPSCs using zinc-finger nucleases. Nat Biotechnol. 2009; 27:851–857. [PubMed: 19680244]

Hockemeyer D, Wang H, Kiani S, Lai CS, Gao Q, Cassady JP, Cost GJ, Zhang L, Santiago Y, Miller JC, Zeitler B, Cherone JM, Meng X, Hinkley SJ, Rebar EJ, Gregory PD, Urnov FD, Jaenisch R. Genetic engineering of human pluripotent cells using TALE nucleases. Nature biotechnology. 2011; 29:731–734.

Holt N, Wang J, Kim K, Friedman G, Wang X, Taupin V, Crooks GM, Kohn DB, Gregory PD, Holmes MC, Cannon PM. Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 in vivo. Nature biotechnology. 2010; 28:839–847.

Hong K, Sherley J, Lauffenburger DA. Methylation of episomal plasmids as a barrier to transient gene expression via a synthetic delivery vector. Biomolecular engineering. 2001; 18:185–192. [PubMed: 11576873]

Huertas P, Jackson SP. Human CtIP mediates cell cycle control of DNA end resection and double strand break repair. J Biol Chem. 2009; 284:9558–9565. [PubMed: 19202191]

Jarjour J, West-Foyle H, Certo MT, Hubert CG, Doyle L, Getz MM, Stoddard BL, Scharenberg AM. High-resolution profiling of homing endonuclease binding and catalytic specificity using yeast surface display. Nucleic Acids Res. 2009; 37:6871–6880. [PubMed: 19740766]

Kantor B, Ma H, Webster-Cyriaque J, Monahan PE, Kafri T. Epigenetic activation of unintegrated HIV-1 genomes by gut-associated short chain fatty acids and its implications for HIV infection. Proc Natl Acad Sci U S A. 2009; 106:18786–18791. [PubMed: 19843699]

Kayali R, Bury F, Ballard M, Bertoni C. Site-directed gene repair of the dystrophin gene mediated by PNA-ssODNs. Hum Mol Genet. 2010; 19:3266–3281. [PubMed: 20542988]

Kim HJ, Lee HJ, Kim H, Cho SW, Kim JS. Targeted genome editing in human cells with zinc finger nucleases constructed via modular assembly. Genome research. 2009; 19:1279–1288. [PubMed: 19470664]

Kim JA, Kruhlak M, Dotiwala F, Nussenzweig A, Haber JE. Heterochromatin is refractory to gamma-H2AX modification in yeast and mammals. J Cell Biol. 2007a; 178:209–218. [PubMed: 17635934]

Kim KH, Nielsen PE, Glazer PM. Site-directed gene mutation at mixed sequence targets by psoralen-conjugated pseudo-complementary peptide nucleic acids. Nucleic Acids Res. 2007b; 35:7604–7613. [PubMed: 17977869]

Kim S, Lee MJ, Kim H, Kang M, Kim JS. Preassembled zinc-finger arrays for rapid construction of ZFNs. Nat Methods. 2011; 8:7. [PubMed: 21191366]

Kim YG, Cha J, Chandrasegaran S. Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. Proc Natl Acad Sci U S A. 1996; 93:1156–1160. [PubMed: 8577732]

Klug A. The discovery of zinc fingers and their applications in gene regulation and genome manipulation. Annu Rev Biochem. 2010; 79:213–231. [PubMed: 20192761]

Larocque JR, Jasin M. Mechanisms of recombination between diverged sequences in wild-type and BLM-deficient mouse and human cells. Mol Cell Biol. 2010; 30:1887–1897. [PubMed: 20154148]

Larocque JR, Stark JM, Oh J, Bojilova E, Yusa K, Horie K, Takeda J, Jasin M. Interhomolog recombination and loss of heterozygosity in wild-type and Bloom syndrome helicase (BLM)-deficient mammalian cells. Proc Natl Acad Sci U S A. 2011; 108:11971–11976. [PubMed: 21730139]

Lee GS, Neiditch MB, Salus SS, Roth DB. RAG proteins shepherd double-strand breaks to a specific pathway, suppressing error-prone repair, but RAG nicking initiates homologous recombination. Cell. 2004; 117:171–184. [PubMed: 15084256]

Lee HJ, Kim E, Kim JS. Targeted chromosomal deletions in human cells using zinc finger nucleases. Genome Res. 2010; 20:81–89. [PubMed: 19952142]

Li T, Huang S, Zhao X, Wright DA, Carpenter S, Spalding MH, Weeks DP, Yang B. Modularly assembled designer TAL effector nucleases for targeted gene knockout and gene replacement in eukaryotes. Nucleic Acids Res. 2011; 39:6315–6325. [PubMed: 21459844]

Limbo O, Chahwan C, Yamada Y, de Bruin RA, Wittenberg C, Russell P. Ctp1 is a cell-cycle-regulated protein that functions with Mre11 complex to control double-strand break repair by homologous recombination. Mol Cell. 2007; 28:134–146. [PubMed: 17936710]

Liu J, Majumdar A, Thompson LH, Seidman MM. Sequence conversion by single strand oligonucleotide donors via non-homologous end joining in mammalian cells. J Biol Chem. 2010; 285:23198–23207. [PubMed: 20489199]

Liu Y, Nairn RS, Vasquez KM. Targeted gene conversion induced by triplex-directed psoralen interstrand crosslinks in mammalian cells. Nucleic Acids Res. 2009; 37:6378–6388. [PubMed: 19726585]

Llorente B, Smith CE, Symington LS. Break-induced replication: what is it and what is it for? Cell Cycle. 2008; 7:859–864. [PubMed: 18414031]

Lombardo A, Genovese P, Beausejour CM, Colleoni S, Lee YL, Kim KA, Ando D, Urnov FD, Galli C, Gregory PD, Holmes MC, Naldini L. Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery. Nature biotechnology. 2007; 25:1298–1306.

Lonkar P, Kim KH, Kuan JY, Chin JY, Rogers FA, Knauert MP, Kole R, Nielsen PE, Glazer PM. Targeted correction of a thalassemia-associated beta-globin mutation induced by pseudo-complementary peptide nucleic acids. Nucleic Acids Res. 2009; 37:3635–3644. [PubMed: 19364810]

Lorenz A, Whitby MC. Crossover promotion and prevention. Biochem Soc Trans. 2006; 34:537–541. [PubMed: 16856854]

Lukacsovich T, Yang D, Waldman AS. Repair of a specific double-strand break generated within a mammalian chromosome by yeast endonuclease I-SceI. Nucleic Acids Res. 1994; 22:5649–5657. [PubMed: 7838718]

Maeder ML, Thibodeau-Beganny S, Osiak A, Wright DA, Anthony RM, Eichtinger M, Jiang T, Foley JE, Winfrey RJ, Townsend JA, Unger-Wallace E, Sander JD, Muller-Lerch F, Fu F, Pearlberg J, Gobel C, Dassie JP, Pruett-Miller SM, Porteus MH, Sgroi DC, Iafrate AJ, Dobbs D, McCray PB Jr. Cathomen T, Voytas DF, Joung JK. Rapid “open-source” engineering of customized zinc-finger nucleases for highly efficient gene modification. Mol Cell. 2008; 31:294–301. [PubMed: 18657511]

Maguire AM, Simonelli F, Pierce EA, Pugh EN Jr. Mingozzi F, Bennicelli J, Banfi S, Marshall KA, Testa F, Surace EM, Rossi S, Lyubarsky A, Arruda VR, Konkle B, Stone E, Sun J, Jacobs J, Dell'Osso L, Hertle R, Ma JX, Redmond TM, Zhu X, Hauck B, Zelenaia O, Shindler KS, Maguire MG, Wright JF, Volpe NJ, McDonnell JW, Auricchio A, High KA, Bennett J. Safety and efficacy of gene transfer for Leber's congenital amaurosis. N Engl J Med. 2008; 358:2240–2248. [PubMed: 18441370]

Mahaney BL, Meek K, Lees-Miller SP. Repair of ionizing radiation-induced DNA double-strand breaks by non-homologous end-joining. Biochem J. 2009; 417:639–650. [PubMed: 19133841]

Majumdar A, Muniandy PA, Liu J, Liu JL, Liu ST, Cuenoud B, Seidman MM. Targeted gene knock in and sequence modulation mediated by a psoralen-linked triplex-forming oligonucleotide. J Biol Chem. 2008; 283:11244–11252. [PubMed: 18303025]

Malone RW, Felgner PL, Verma IM. Cationic liposome-mediated RNA transfection. Proc Natl Acad Sci U S A. 1989; 86:6077–6081. [PubMed: 2762315]

Manno CS, Pierce GF, Arruda VR, Glader B, Ragni M, Rasko JJ, Ozelo MC, Hoots K, Blatt P, Konkle B, Dake M, Kaye R, Razavi M, Zajko A, Zehnder J, Rustagi PK, Nakai H, Chew A, Leonard D, Wright JF, Lessard RR, Sommer JM, Tigges M, Sabatino D, Luk A, Jiang H, Mingozzi F, Couto L, Ertl HC, High KA, Kay MA. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. Nat Med. 2006; 12:342–347. [PubMed: 16474400]

Matrai J, Chuah MK, VandenDriessche T. Recent advances in lentiviral vector development and applications. Mol Ther. 2011; 18:477–490. [PubMed: 20087315]

McConnell Smith A, Takeuchi R, Pellenz S, Davis L, Maizels N, Monnat RJ Jr. Stoddard BL. Generation of a nicking enzyme that stimulates site-specific gene conversion from the I-AniI LAGLIDADG homing endonuclease. Proc Natl Acad Sci U S A. 2009; 106:5099–5104. [PubMed: 19276110]

McCord RA, Michishita E, Hong T, Berber E, Boxer LD, Kusumoto R, Guan S, Shi X, Gozani O, Burlingame AL, Bohr VA, Chua KF. SIRT6 stabilizes DNA-dependent protein kinase at chromatin for DNA double-strand break repair. Aging (Albany NY). 2009; 1:109–121. [PubMed: 20157594]

Mehnert JM, Kelly WK. Histone deacetylase inhibitors: biology and mechanism of action. Cancer J. 2007; 13:23–29. [PubMed: 17464243]

Mendell JR, Campbell K, Rodino-Klapac L, Sahenk Z, Shilling C, Lewis S, Bowles D, Gray S, Li C, Galloway G, Malik V, Coley B, Clark KR, Li J, Xiao X, Samulski J, McPhee SW, Samulski RJ, Walker CM. Dystrophin immunity in Duchenne's muscular dystrophy. N Engl J Med. 2010; 363:1429–1437. [PubMed: 20925545]

Metzger MJ, McConnell-Smith A, Stoddard BL, Miller AD. Single-strand nicks induce homologous recombination with less toxicity than double-strand breaks using an AAV vector template. Nucleic Acids Res. 2010; 39:926–935. [PubMed: 20876694]

Miller JC, Holmes MC, Wang J, Guschin DY, Lee YL, Rupniewski I, Beausejour CM, Waite AJ, Wang NS, Kim KA, Gregory PD, Pabo CO, Rebar EJ. An improved zinc-finger nuclease architecture for highly specific genome editing. Nat Biotechnol. 2007; 25:778–785. [PubMed: 17603475]

Miller KM, Tjeertes JV, Coates J, Legube G, Polo SE, Britton S, Jackson SP. Human HDAC1 and HDAC2 function in the DNA-damage response to promote DNA nonhomologous end-joining. Nat Struct Mol Biol. 2010; 17:1144–1151. [PubMed: 20802485]

Mitchell AM, Nicolson SC, Warischalk JK, Samulski RJ. AAV's anatomy: roadmap for optimizing vectors for translational success. Curr Gene Ther. 2010; 10:319–340. [PubMed: 20712583]

Mladenov E, Iliakis G. Induction and repair of DNA double strand breaks: the increasing spectrum of non-homologous end joining pathways. Mutat Res. 2011; 711:61–72. [PubMed: 21329706]

Moehle EA, Rock JM, Lee YL, Jouvenot Y, DeKelver RC, Gregory PD, Urnov FD, Holmes MC. Targeted gene addition into a specified location in the human genome using designed zinc finger nucleases. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:3055–3060. [PubMed: 17360608]

Moore MJ, Flotte TR. Autoimmunity in a genetic disease-a cautionary tale. N Engl J Med. 2010; 363:1473–1475. [PubMed: 20925551]

Morbitzer R, Elsaesser J, Hausner J, Lahaye T. Assembly of custom TALE-type DNA binding domains by modular cloning. Nucleic Acids Res. 2011; 39:5790–5799. [PubMed: 21421566]

Moscou MJ, Bogdanove AJ. A simple cipher governs DNA recognition by TAL effectors. Science. 2009; 326:1501. [PubMed: 19933106]

Moynahan ME, Jasin M. Mitotic homologous recombination maintains genomic stability and suppresses tumorigenesis. Nat Rev Mol Cell Biol. 2010; 11:196–207. [PubMed: 20177395]

Mukherjee A, Vasquez KM. Triplex technology in studies of DNA damage, DNA repair, and mutagenesis. Biochimie. 2011; 93:1197–1208. [PubMed: 21501652]

Mussolino C, Morbitzer R, Lutge F, Dannemann N, Lahaye T, Cathomen T. A novel TALE nuclease scaffold enables high genome editing activity in combination with low toxicity. Nucleic acids research. 2011

Nagaraju G, Hartlerode A, Kwok A, Chandramouly G, Scully R. XRCC2 and XRCC3 regulate the balance between short- and long-tract gene conversions between sister chromatids. Mol Cell Biol. 2009; 29:4283–4294. [PubMed: 19470754]

Nagaraju G, Odate S, Xie A, Scully R. Differential regulation of short- and long-tract gene conversion between sister chromatids by Rad51C. Mol Cell Biol. 2006; 26:8075–8086. [PubMed: 16954385]

Narsinh KH, Wu JC. Gene correction in human embryonic and induced pluripotent stem cells: promises and challenges ahead. Mol Ther. 2010; 18:1061–1063. [PubMed: 20514030]

Nickoloff JA, Chen EY, Heffron F. A 24-base-pair DNA sequence from the MAT locus stimulates intergenic recombination in yeast. Proc Natl Acad Sci U S A. 1986; 83:7831–7835. [PubMed: 3020559]

Nimonkar AV, Genschel J, Kinoshita E, Polaczek P, Campbell JL, Wyman C, Modrich P, Kowalczykowski SC. BLM-DNA2-RPA-MRN and EXO1-BLM-RPA-MRN constitute two DNA end resection machineries for human DNA break repair. Genes Dev. 2011; 25:350–362. [PubMed: 21325134]

Niu Y, Tenney K, Li H, Gimble FS. Engineering variants of the I-SceI homing endonuclease with strand-specific and site-specific DNA-nicking activity. J Mol Biol. 2008; 382:188–202. [PubMed: 18644379]

Nussenzweig A, Nussenzweig MC. Origin of chromosomal translocations in lymphoid cancer. Cell. 2010; 141:27–38. [PubMed: 20371343]

O'Keefe C, McDevitt MA, Maciejewski JP. Copy neutral loss of heterozygosity: a novel chromosomal lesion in myeloid malignancies. Blood. 2010; 115:2731–2739. [PubMed: 20107230]

Odersky A, Panyutin IV, Panyutin IG, Schunck C, Feldmann E, Goedecke W, Neumann RD, Obe G, Pfeiffer P. Repair of sequence-specific 125I-induced double-strand breaks by nonhomologous DNA end joining in mammalian cell-free extracts. J Biol Chem. 2002; 277:11756–11764. [PubMed: 11821407]

Okada T, Uchibori R, Iwata-Okada M, Takahashi M, Nomoto T, Nonaka-Sarukawa M, Ito T, Liu Y, Mizukami H, Kume A, Kobayashi E, Ozawa K. A histone deacetylase inhibitor enhances recombinant adeno-associated virus-mediated gene expression in tumor cells. Mol Ther. 2006; 13:738–746. [PubMed: 16387551]

Ordinario EC, Yabuki M, Handa P, Cummings WJ, Maizels N. RAD51 paralogs promote homology-directed repair at diversifying immunoglobulin V regions. BMC Mol Biol. 2009; 10:98. [PubMed: 19863810]

Papapetrou EP, Lee G, Malani N, Setty M, Riviere I, Tirunagari LM, Kadota K, Roth SL, Giardina P, Viale A, Leslie C, Bushman FD, Studer L, Sadelain M. Genomic safe harbors permit high beta-globin transgene expression in thalassemia induced pluripotent stem cells. Nat Biotechnol. 2011; 29:73–78. [PubMed: 21151124]

Pattanayak V, Ramirez CL, Joung JK, Liu DR. Revealing off-target cleavage specificities of zinc-finger nucleases by in vitro selection. Nat Methods. 2011; 8:765–770. [PubMed: 21822273]

Perez EE, Wang J, Miller JC, Jouvenot Y, Kim KA, Liu O, Wang N, Lee G, Bartsevich VV, Lee YL, Guschin DY, Rupniewski I, Waite AJ, Carpenito C, Carroll RG, Orange JS, Urnov FD, Rebar EJ, Ando D, Gregory PD, Riley JL, Holmes MC, June CH. Establishment of HIV-1 resistance in CD4+ T cells by genome editing using zinc-finger nucleases. Nature biotechnology. 2008; 26:808–816.

Petek LM, Russell DW, Miller DG. Frequent endonuclease cleavage at off-target locations in vivo. Mol Ther. 2010; 18:983–986. [PubMed: 20216527]

Pichavant C, Aartsma-Rus A, Clemens PR, Davies KE, Dickson G, Takeda S, Wilton SD, Wolff JA, Wooddell CI, Xiao X, Tremblay JP. Current status of pharmaceutical and genetic therapeutic approaches to treat DMD. Mol Ther. 2011; 19:830–840. [PubMed: 21468001]

Plessis A, Perrin A, Haber JE, Dujon B. Site-specific recombination determined by I-SceI, a mitochondrial group I intron-encoded endonuclease expressed in the yeast nucleus. Genetics. 1992; 130:451–460. [PubMed: 1551570]

Polo SE, Jackson SP. Dynamics of DNA damage response proteins at DNA breaks: a focus on protein modifications. Genes Dev. 2011; 25:409–433. [PubMed: 21363960]

Porteus MH. Mammalian gene targeting with designed zinc finger nucleases. Mol Ther. 2006; 13:438–446. [PubMed: 16169774]

Porteus MH, Baltimore D. Chimeric nucleases stimulate gene targeting in human cells. Science. 2003; 300:763. [PubMed: 12730593]

Porteus MH, Carroll D. Gene targeting using zinc finger nucleases. Nat Biotechnol. 2005; 23:967–973. [PubMed: 16082368]

Porteus MH, Cathomen T, Weitzman MD, Baltimore D. Efficient gene targeting mediated by adeno-associated virus and DNA double-strand breaks. Mol Cell Biol. 2003; 23:3558–3565. [PubMed: 12724414]

Pruett-Miller SM, Connelly JP, Maeder ML, Joung JK, Porteus MH. Comparison of zinc finger nucleases for use in gene targeting in mammalian cells. Molecular therapy : the journal of the American Society of Gene Therapy. 2008; 16:707–717. [PubMed: 18334988]

Pruett-Miller SM, Reading DW, Porter SN, Porteus MH. Attenuation of zinc finger nuclease toxicity by small-molecule regulation of protein levels. PLoS Genet. 2009; 5:e1000376. [PubMed: 19214211]

Puchta H, Dujon B, Hohn B. Homologous recombination in plant cells is enhanced by in vivo induction of double strand breaks into DNA by a site-specific endonuclease. Nucleic Acids Res. 1993; 21:5034–5040. [PubMed: 8255757]

Raab JR, Kamakaka RT. Insulators and promoters: closer than we think. Nat Rev Genet. 2010; 11:439–446. [PubMed: 20442713]

Radecke F, Peter I, Radecke S, Gellhaus K, Schwarz K, Cathomen T. Targeted chromosomal gene modification in human cells by single-stranded oligodeoxynucleotides in the presence of a DNA double-strand break. Mol Ther. 2006; 14:798–808. [PubMed: 16904944]

Radecke S, Radecke F, Cathomen T, Schwarz K. Zinc-finger nuclease-induced gene repair with oligodeoxynucleotides: wanted and unwanted target locus modifications. Mol Ther. 2010; 18:743–753. [PubMed: 20068556]

Rahman SH, Maeder ML, Joung JK, Cathomen T. Zinc-finger nucleases for somatic gene therapy: the next frontier. Hum Gene Ther. 2011; 22:925–933. [PubMed: 21631241]

Ramalingam S, Kandavelou K, Rajenderan R, Chandrasegaran S. Creating designed zinc-finger nucleases with minimal cytotoxicity. J Mol Biol. 2011; 405:630–641. [PubMed: 21094162]

Redondo P, Prieto J, Munoz IG, Alibes A, Stricher F, Serrano L, Cabaniols JP, Daboussi F, Arnould S, Perez C, Duchateau P, Paques F, Blanco FJ, Montoya G. Molecular basis of xeroderma pigmentosum group C DNA recognition by engineered meganucleases. Nature. 2008; 456:107–111. [PubMed: 18987743]

Reeves R, Gorman CM, Howard B. Minichromosome assembly of non-integrated plasmid DNA transfected into mammalian cells. Nucleic Acids Res. 1985; 13:3599–3615. [PubMed: 3859838]

Rouet P, Smih F, Jasin M. Expression of a site-specific endonuclease stimulates homologous recombination in mammalian cells. Proc Natl Acad Sci U S A. 1994a; 91:6064–6068. [PubMed: 8016116]

Rouet P, Smih F, Jasin M. Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease. Mol Cell Biol. 1994b; 14:8096–8106. [PubMed: 7969147]

Russell DW, Hirata RK. Human gene targeting by viral vectors. Nat Genet. 1998; 18:325–330. [PubMed: 9537413]

Sander JD, Maeder ML, Joung JK. Engineering designer nucleases with customized cleavage specificities. Curr Protoc Mol Biol Chapter. 2011; 12 Unit12 13.

Sanders KL, Catto LE, Bellamy SR, Halford SE. Targeting individual subunits of the FokI restriction endonuclease to specific DNA strands. Nucleic Acids Res. 2009; 37:2105–2115. [PubMed: 19223323]

Sartori AA, Lukas C, Coates J, Mistrik M, Fu S, Bartek J, Baer R, Lukas J, Jackson SP. Human CtIP promotes DNA end resection. Nature. 2007; 450:509–514. [PubMed: 17965729]

Scalley-Kim M, McConnell-Smith A, Stoddard BL. Coevolution of a homing endonuclease and its host target sequence. J Mol Biol. 2007; 372:1305–1319. [PubMed: 17720189]

Shaw KL, Kohn DB. A tale of two SCIDs. Sci Transl Med. 2011; 3:97ps36.

Simsek D, Brunet E, Wong SY, Katyal S, Gao Y, McKinnon PJ, Lou J, Zhang L, Li J, Rebar EJ, Gregory PD, Holmes MC, Jasin M. DNA ligase III promotes alternative nonhomologous end-joining during chromosomal translocation formation. PLoS Genet. 2011; 7:e1002080. [PubMed: 21655080]

Simsek D, Jasin M. Alternative end-joining is suppressed by the canonical NHEJ component Xrcc4-ligase IV during chromosomal translocation formation. Nat Struct Mol Biol. 2010; 17:410–416. [PubMed: 20208544]

Smith CE, Llorente B, Symington LS. Template switching during break-induced replication. Nature. 2007; 447:102–105. [PubMed: 17410126]

Soldner F, Laganiere J, Cheng AW, Hockemeyer D, Gao Q, Alagappan R, Khurana V, Golbe LI, Myers RH, Lindquist S, Zhang L, Guschin D, Fong LK, Vu BJ, Meng X, Urnov FD, Rebar EJ, Gregory PD, Zhang HS, Jaenisch R. Generation of isogenic pluripotent stem cells differing exclusively at two early onset Parkinson point mutations. Cell. 2011; 146:318–331. [PubMed: 21757228]

Sollu C, Pars K, Cornu TI, Thibodeau-Beganny S, Maeder ML, Joung JK, Heilbronn R, Cathomen T. Autonomous zinc-finger nuclease pairs for targeted chromosomal deletion. Nucleic acids research. 2010; 38:8269–8276. [PubMed: 20716517]

Soutoglou E, Misteli T. Activation of the cellular DNA damage response in the absence of DNA lesions. Science. 2008; 320:1507–1510. [PubMed: 18483401]

Stein S, Ott MG, Schultze-Strasser S, Jauch A, Burwinkel B, Kinner A, Schmidt M, Kramer A, Schwable J, Glimm H, Koehl U, Preiss C, Ball C, Martin H, Gohring G, Schwarzwaelder K, Hofmann WK, Karakaya K, Tchatchou S, Yang R, Reinecke P, Kuhlcke K, Schlegelberger B, Thrasher AJ, Hoelzer D, Seger R, von Kalle C, Grez M. Genomic instability and myelodysplasia with monosomy 7 consequent to EVI1 activation after gene therapy for chronic granulomatous disease. Nat Med. 2010; 16:198–204. [PubMed: 20098431]

Storici F, Durham CL, Gordenin DA, Resnick MA. Chromosomal site-specific double-strand breaks are efficiently targeted for repair by oligonucleotides in yeast. Proc Natl Acad Sci U S A. 2003; 100:14994–14999. [PubMed: 14630945]

Strathern JN, Weinstock KG, Higgins DR, McGill CB. A novel recombinator in yeast based on gene II protein from bacteriophage f1. Genetics. 1991; 127:61–73. [PubMed: 2016047]

Symington LS, Gautier J. Double-strand break end resection and repair pathway choice. Annu Rev Genet. 2010

Szczepek M, Brondani V, Buchel J, Serrano L, Segal DJ, Cathomen T. Structure-based redesign of the dimerization interface reduces the toxicity of zinc-finger nucleases. Nat Biotechnol. 2007; 25:786–793. [PubMed: 17603476]

Szeto MD, Boissel SJ, Baker D, Thyme SB. Mining endonuclease cleavage determinants in genomic sequence data. J Biol Chem. 2011; 286:32617–32627. [PubMed: 21778233]

Taghian DG, Nickoloff JA. Chromosomal double-strand breaks induce gene conversion at high frequency in mammalian cells. Mol Cell Biol. 1997; 17:6386–6393. [PubMed: 9343400]

Takeuchi R, Lambert AR, Mak AN, Jacoby K, Dickson RJ, Gloor GB, Scharenberg AM, Edgell DR, Stoddard BL. Tapping natural reservoirs of homing endonucleases for targeted gene modification. Proc Natl Acad Sci U S A. 2011; 108:13077–13082. [PubMed: 21784983]

Thompson SL, Compton DA. Chromosomes and cancer cells. Chromosome Res. 2010; 19:433–444. [PubMed: 21190130]

Thyme SB, Jarjour J, Takeuchi R, Havranek JJ, Ashworth J, Scharenberg AM, Stoddard BL, Baker D. Exploitation of binding energy for catalysis and design. Nature. 2009; 461:1300–1304. [PubMed: 19865174]

Urnov FD, Miller JC, Lee YL, Beausejour CM, Rock JM, Augustus S, Jamieson AC, Porteus MH, Gregory PD, Holmes MC. Highly efficient endogenous human gene correction using designed zinc-finger nucleases. Nature. 2005; 435:646–651. [PubMed: 15806097]

Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD. Genome editing with engineered zinc finger nucleases. Nat Rev Genet. 2011; 11:636–646. [PubMed: 20717154]

Van Tendeloo VF, Ponsaerts P, Lardon F, Nijs G, Lenjou M, Van Broeckhoven C, Van Bockstaele DR, Berneman ZN. Highly efficient gene delivery by mRNA electroporation in human hematopoietic cells: superiority to lipofection and passive pulsing of mRNA and to electroporation of plasmid cDNA for tumor antigen loading of dendritic cells. Blood. 2001; 98:49–56. [PubMed: 11418462]

Wang H, Perrault AR, Takeda Y, Qin W, Iliakis G. Biochemical evidence for Ku-independent backup pathways of NHEJ. Nucleic Acids Res. 2003; 31:5377–5388. [PubMed: 12954774]

Wanisch K, Yanez-Munoz RJ. Integration-deficient lentiviral vectors: a slow coming of age. Mol Ther. 2009; 17:1316–1332. [PubMed: 19491821]

Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, Ebina W, Mandal PK, Smith ZD, Meissner A, Daley GQ, Brack AS, Collins JJ, Cowan C, Schlaeger TM, Rossi DJ. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell. 2010; 7:618–630. [PubMed: 20888316]

Yabuki M, Ordinario EC, Cummings WJ, Fujii MM, Maizels N. E2A acts in cis in G1 phase of cell cycle to promote Ig gene diversification. J Immunol. 2009; 182:408–415. [PubMed: 19109172]

Yanez-Munoz RJ, Balaggan KS, MacNeil A, Howe SJ, Schmidt M, Smith AJ, Buch P, MacLaren RE, Anderson PN, Barker SE, Duran Y, Bartholomae C, von Kalle C, Heckenlively JR, Kinnon C, Ali RR, Thrasher AJ. Effective gene therapy with nonintegrating lentiviral vectors. Nat Med. 2006; 12:348–353. [PubMed: 16491086]

Yannaki E, Emery DW, Stamatoyannopoulos G. Gene therapy for beta-thalassaemia: the continuing challenge. Expert Rev Mol Med. 2010; 12:e31. [PubMed: 20883576]

Yun MH, Hiom K. CtIP-BRCA1 modulates the choice of DNA double-strand-break repair pathway throughout the cell cycle. Nature. 2009; 459:460–463. [PubMed: 19357644]

Zhang F, Cong L, Lodato S, Kosuri S, Church GM, Arlotta P. Efficient construction of sequence-specific TAL effectors for modulating mammalian transcription. Nat Biotechnol. 2011; 29:149–153. [PubMed: 21248753]

Zhao N, Fogg JM, Zechiedrich L, Zu Y. Transfection of shRNA-encoding Minivector DNA of a few hundred base pairs to regulate gene expression in lymphoma cells. Gene Ther. 2010; 18:220–224. [PubMed: 20962872]

Zou J, Maeder ML, Mali P, Pruett-Miller SM, Thibodeau-Beganny S, Chou BK, Chen G, Ye Z, Park IH, Daley GQ, Porteus MH, Joung JK, Cheng L. Gene targeting of a disease-related gene in human induced pluripotent stem and embryonic stem cells. Cell Stem Cell. 2009; 5:97–110. [PubMed: 19540188]

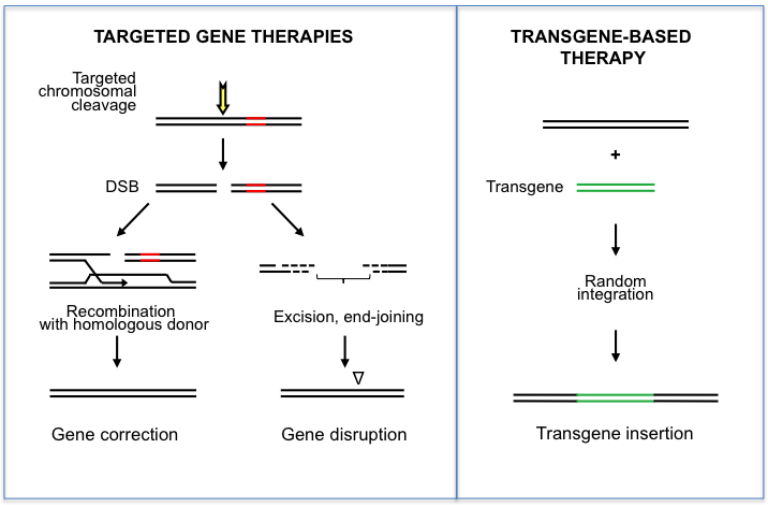

Figure 1. Targeted gene therapies and transgene-based therapies

Targeted gene therapies (left) enlist the cell's DNA repair pathways to correct or disrupt a gene of interest. In the first step, the chromosome is cleaved by a rare-cutting endonuclease, to generate a double-strand break (DSB) as shown, or a DNA nick. DNA repair by homologous recombination with an exogenous donor template can result in targeted gene correction, at either a DSB or nick. A DSB can also undergo repair via the nonhomologous end-joining pathway, creating a short deletion that inactivates gene function to effect targeted gene disruption. Transgene-based therapies (right) provide a therapeutic gene typically utilizing viral vectors. Integration of the transgene is typically not targeted to a specific locus and can result in insertional mutagenesis. A color version of this figure is available online.

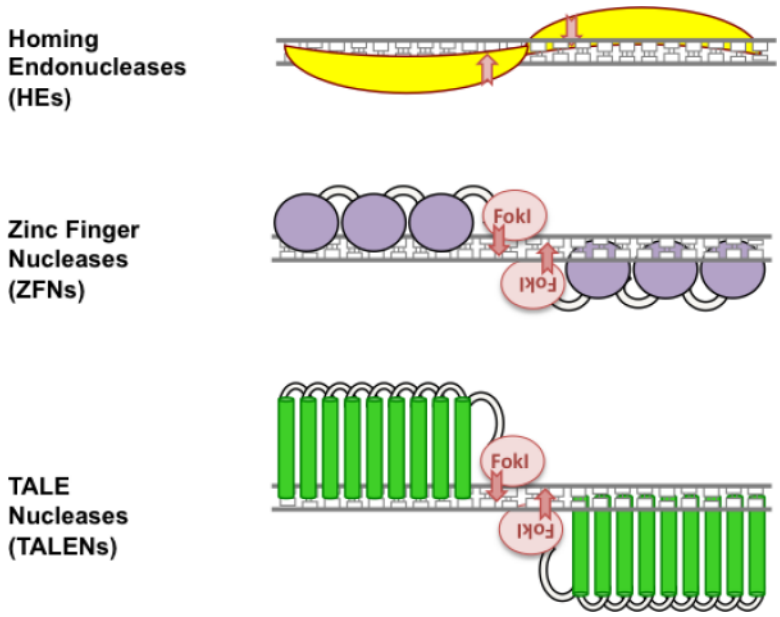

Figure 2. Domain structures of HEs, ZFNs and TALENs

Diagrams of domain structures of the rare-cutting endonucleases currently in use for gene therapies. Homing endonucleases (HEs) may be monomeric or dimeric, with two active sites that cleave top and bottom DNA strands (arrows). Zinc finger nucleases (ZFNs) are engineered to include domains that recognize 3 bp regions of DNA (circles), joined by linkers and fused to the FokI restriction nuclease domain, to cleave the central region on the top and bottom DNA strands (arrows). TALE nucleases (TALENs) are engineered to include domains that recognize a single DNA base pair (cylinders), joined by linkers and fused to the FokI restriction nuclease domain, to cleave the central region on the top and bottom DNA strands (arrows). A color version of this figure is available online.

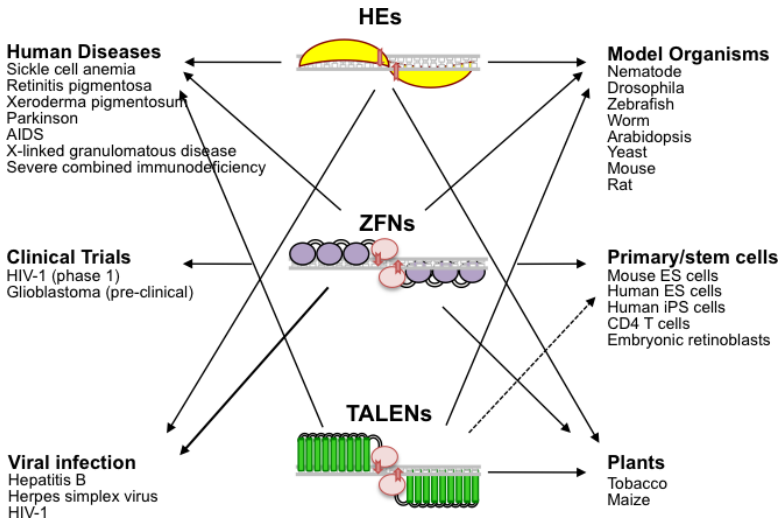

Figure 3. Targeted genome engineering applications using HEs, ZFNs and TALENs

Applications of HEs, ZFNs and TALENs in genome engineering. These nucleases have been employed to target a variety of organisms, cell types, viruses, and genes implicated in human diseases, as shown. ZFNs have been used the most extensively in genome engineering applications, but HEs and TALENs are rapidly catching up. A color version of this figure is available online. A more detailed list of the genes and organisms targeted by these endonucleases is presented in Supplementary Table 1.

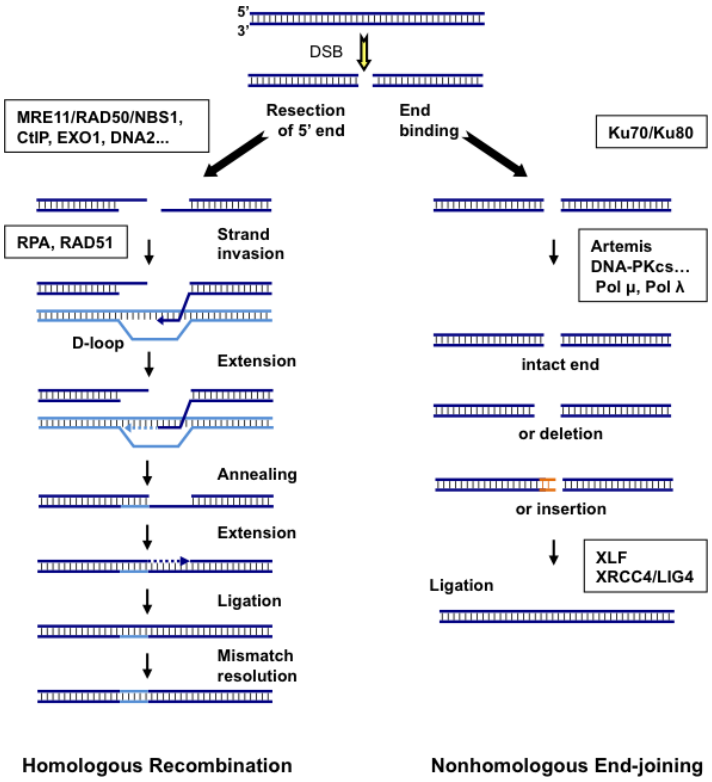

Figure 4. Alternate pathways of DNA DSB repair

Homologous recombination (left) and nonhomologous end joining (right) are the two major modes of repair at a DNA DSB. In order to enter the homologous recombination pathway, a DSB must undergo extensive 5'-end resection to leave free 3'-ends that can invade the repair template, to form a D-loop (left). In the predominate homologous recombination sub-pathway in somatic cells, synthesis-dependent strand annealing, the invading 3' end then primes limited replication using the homologous DNA as template. The newly extended end is then released from the D-loop and anneals with the other end of the processed DSB. Synthesis-dependent strand annealing transfers relatively short stretches of DNA sequence. After ligation, the resulting heteroduplex is resolved either by the mismatch repair pathway or replication and segregation to generate a corrected gene. Alternatively, a DNA DSB may enter the nonhomologous end-joining pathway (right), in which DNA ends are bound by the Ku70-Ku80 heterodimer. Limited processing occurs, which may be accompanied by small insertions or deletions, and generates DNA ends that can be ligated by the XRCC4-LIG4 complex. The factors that play important roles at various stages of these DSB repair pathways are shown in boxes. A color version of this figure is available online.

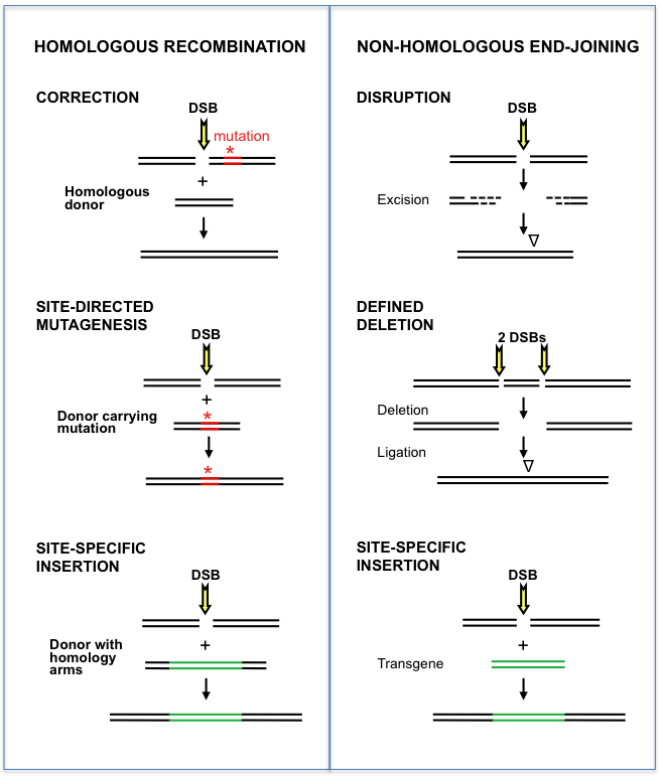

Figure 5. Variations of targeted genome engineering

This diagram illustrates some of the outcomes that can result when a targeted DNA double-strand break (DSB) is repaired by homologous recombination or nonhomologous end joining. Homologous recombination (left) requires a donor homologous at least in part with the cleaved allele. Depending on details of that donor, it may be used to effect gene correction, site-directed mutagenesis, or site-specific insertion driven by matching arms of homology. Nonhomologous end joining (right) will occur in the absence of donor DNA. If cleavage is followed by excision, genetic information may be lost (or gained) at the site of the break, and gene disruption can result. Two DSBs occurring simultaneously on the same chromosome can cause loss of the intervening stretch of DNA, resulting in a defined deletion. Targeted cleavage may also lead to site-specific insertion of a transgene at a defined DSB. A color version of this figure is available online.