

METHODS AND COMPOSITIONS FOR TARGETED CLEAVAGE AND RECOMBINATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 12/804,234, filed Jul. 16, 2010, which is a continuation of U.S. patent application Ser. No. 10/912,932 filed Aug. 6, 2004, now U.S. Pat. No. 7,888,121, which claims the benefit of the following U.S. provisional patent applications: 60/493,931 filed Aug. 8, 2003; 60/518,253 filed Nov. 7, 2003; 60/530,541 filed Dec. 18, 2003; 60/542,780 filed Feb. 5, 2004; 60/556,831 filed Mar. 26, 2004 and 60/575,919 filed Jun. 1, 2004; the disclosures of which are incorporated by reference in their entireties for all purposes.

STATEMENT OF RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH

[0002] Not applicable.

TECHNICAL FIELD

[0003] The present disclosure is in the field of genome engineering and homologous recombination.

BACKGROUND

[0004] A major area of interest in genome biology, especially in light of the determination of the complete nucleotide sequences of a number of genomes, is the targeted alteration of genome sequences. To provide but one example, sickle cell anemia is caused by mutation of a single nucleotide pair in the human β -globin gene. Thus, the ability to convert the endogenous genomic copy of this mutant nucleotide pair to the wild-type sequence in a stable fashion and produce normal β -globin would provide a cure for sickle cell anemia.

[0005] Attempts have been made to alter genomic sequences in cultured cells by taking advantage of the natural phenomenon of homologous recombination. See, for example, Capecchi (1989) *Science* 244:1288-1292; U.S. Pat. Nos. 6,528,313 and 6,528,314. If a polynucleotide has sufficient homology to the genomic region containing the sequence to be altered, it is possible for part or all of the sequence of the polynucleotide to replace the genomic sequence by homologous recombination. However, the frequency of homologous recombination under these circumstances is extremely low. Moreover, the frequency of insertion of the exogenous polynucleotide at genomic locations that lack sequence homology exceeds the frequency of homologous recombination by several orders of magnitude.

[0006] The introduction of a double-stranded break into genomic DNA, in the region of the genome bearing homology to an exogenous polynucleotide, has been shown to stimulate homologous recombination at this site by several thousand-fold in cultured cells. Rouet et al. (1994) *Mol. Cell. Biol.* 14:8096-8106; Choulika et al. (1995) *Mol. Cell. Biol.* 15:1968-1973; Donoho et al. (1998) *Mol. Cell. Biol.* 18:4070-4078. See also Johnson et al. (2001) *Biochem. Soc. Trans.* 29:196-201; and Yanez et al. (1998) *Gene Therapy* 5:149-159. In these methods, DNA cleavage in the desired genomic region was accomplished by inserting a recognition site for a meganuclease (i.e., an endonuclease whose recognition

sequence is so large that it does not occur, or occurs only rarely, in the genome of interest) into the desired genomic region.

[0007] However, meganuclease cleavage-stimulated homologous recombination relies on either the fortuitous presence of, or the directed insertion of, a suitable meganuclease recognition site in the vicinity of the genomic region to be altered. Since meganuclease recognition sites are rare (or nonexistent) in a typical mammalian genome, and insertion of a suitable meganuclease recognition site is plagued with the same difficulties as associated with other genomic alterations, these methods are not broadly applicable.

[0008] Thus, there remains a need for compositions and methods for targeted alteration of sequences in any genome.

SUMMARY

[0009] The present disclosure provides compositions and methods for targeted cleavage of cellular chromatin in a region of interest and/or homologous recombination at a predetermined region of interest in cells. Cells include cultured cells, cells in an organism and cells that have been removed from an organism for treatment in cases where the cells and/or their descendants will be returned to the organism after treatment. A region of interest in cellular chromatin can be, for example, a genomic sequence or portion thereof. Compositions include fusion polypeptides comprising an engineered zinc finger binding domain (e.g., a zinc finger binding domain having a novel specificity) and a cleavage domain, and fusion polypeptides comprising an engineered zinc finger binding domain and a cleavage half-domain. Cleavage domains and cleavage half domains can be obtained, for example, from various restriction endonucleases and/or homing endonucleases.

[0010] Cellular chromatin can be present in any type of cell including, but not limited to, prokaryotic and eukaryotic cells, fungal cells, plant cells, animal cells, mammalian cells, primate cells and human cells.

[0011] In one aspect, a method for cleavage of cellular chromatin in a region of interest (e.g., a method for targeted cleavage of genomic sequences) is provided, the method comprising: (a) selecting a first sequence in the region of interest; (b) engineering a first zinc finger binding domain to bind to the first sequence; and (c) expressing a first fusion protein in the cell, the first fusion protein comprising the first engineered zinc finger binding domain and a cleavage domain; wherein the first fusion protein binds to the first sequence and the cellular chromatin is cleaved in the region of interest. The site of cleavage can be coincident with the sequence to which the fusion protein binds, or it can be adjacent (e.g., separated from the near edge of the binding site by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more nucleotides). A fusion protein can be expressed in a cell, e.g., by delivering the fusion protein to the cell or by delivering a polynucleotide encoding the fusion protein to a cell, wherein the polynucleotide, if DNA, is transcribed, and an RNA molecule delivered to the cell or a transcript of a DNA molecule delivered to the cell is translated, to generate the fusion protein. Methods for polynucleotide and polypeptide delivery to cells are presented elsewhere in this disclosure.

[0012] In certain embodiments, the cleavage domain may comprise two cleavage half-domains that are covalently linked in the same polypeptide. The two cleavage half-domains can be derived from the same endonuclease or from different endonucleases.