DNA in atypical locations, including the cytoplasm and endosomes, is understood to trigger immune activation. The presence of DNA anywhere other than the nucleus is thought to trigger DNA recognition systems to detect both DNA genomes of invading pathogens and incorrectly working cells.

Unmethylated CpG DNA motifs, which are abundant in the genomes of many pathogens, are known to be able to stimulate immune responses. Single-stranded DNAs with specific signatures, including AT-rich stem loop regions are also known to activate immune responses.

Therefore, it is desirable to deliver single stranded nucleic acid in a "covert" way, by delivering the linear single stranded nucleic acid in a vector that is not as easily recognised by the innate immunity systems, and then releasing the linear single strand within the cell.

The present inventors have devised a delivery vector that enables the release of a single strand of nucleic acid under appropriate conditions, such as in the nucleus or other compartment of a target cell. The single stranded nucleic acid may be delivered for any conceivable purpose wherein a linear single stranded nucleic acid is desirable, for example as a donor for gene editing, for translation in the cell, for modifying gene expression, for provision of entities such as aptamers and nucleic acid enzymes or for antisense applications.

Summary of the Invention

The present disclosure~~invention~~ relates to a delivery vector, for the delivery of a linear single stranded nucleic acid to a cell, preferably a target cell. The delivery vector is effectively a carrier or delivery vehicle into which the single stranded nucleic acid is placed. Processes within the cell can exploit the structure of the delivery vehicle and release the single stranded nucleic acid. The construct can be manipulated or designed such that the release of the single stranded nucleic acid occurs only under particular conditions or cell types.

The provision of a delivery vector for a linear single stranded nucleic acid permits the covert delivery of an entity that is otherwise quickly degraded. The delivery can be controlled or directed.

According to one aspect, the present ~~invention~~disclosure provides:

a nucleic acid delivery vector comprising a circular single stranded polynucleotide said vector comprising:

(a) a duplex formed from a first section and a third section of said polynucleotide, said sections including sequences which are complementary;

(b) a loop formed from a second section, said section separating the first and third sections;

wherein said duplex includes a recognition sequence for a targeted nuclease.

The nucleic acid delivery vector is described as a single stranded polynucleotide, since under denaturing conditions, the delivery vector is a closed circular polynucleotide.

The delivery vector preferably delivers a linear single stranded nucleic acid. Said single stranded nucleic acid is present within the second section of the delivery vector and the sequence of the second section and the linear single stranded nucleic acid are thus substantially the same. The linear single stranded nucleic acid may take any appropriate conformation and be of any appropriate sequence. The linear single stranded nucleic acid may be a nucleic acid enzyme, an aptamer, a donor template or an antisense nucleic acid, or any of the single stranded nucleic acids discussed herein. The linear single stranded nucleic acid has a free 5' and 3' end, once released from the delivery vector. Depending on the nature of the nuclease, the single stranded nucleic acid may be released with fragments of the first and third sections still present. This is the case for nucleases such as Cas9, and is depicted in Figure 1A and 1B. These fragments may be small, preferably less than 15 bases in length.

The targeted nuclease may be any nuclease that is target specific. The duplex of the delivery vector includes a recognition site for a targeted nuclease. This nuclease may recognise this sequence independently or it may require the help of a guide sequence. The action of the nuclease on the recognition site is the cleavage of the duplex, or at least a strand thereof. Said cleavage may be a blunt cleavage or a staggered cut. The nuclease can be endogenous or exogenous, and if it is the latter, this is also supplied to the cell. The nuclease can be delivered to the cell separately, or it can be delivered by including the mRNA sequence or DNA gene sequence for the nuclease in the delivery vehicle. Should the latter delivery vector be supplied, in effect it provides its own nuclease for releasing the single stranded nucleic acid. The nuclease is preferably an endonuclease. The nuclease may be any entity that can appropriately cleave a phosphodiester bond in a sequence specific manner.

The delivery vector may be used in a cell. The delivery vector may be used to provide a donor template for genome editing. The delivery vector may be used to deliver antisense nucleic acid to a cell. The delivery vector may be used to deliver a nucleic acid enzyme or aptamer to a cell. The delivery vector can be used to deliver any single stranded RNA or nucleic acid hybrid to the cell.

As used herein the cell may be a mammalian cell, preferably a human cell and preferably a human somatic cell.

According to ~~another aspect~~one example, the present ~~invention~~disclosure provides a method of providing a linear single stranded nucleic acid to a cell, comprising the use of a delivery vector as herein described.

According to another aspect, the present disclosure provides an *ex vivo* method of providing a linear single stranded nucleic acid to a cell, comprising the use of a delivery vector as herein described.

According to another aspect, the present disclosure provides a nucleic acid delivery vector as described herein, wherein said vector is for use in therapy.

According to another ~~aspect~~example, the present ~~invention~~disclosure provides a method of providing a linear single stranded donor oligonucleotide or template to a cell for genome editing, comprising the use of a delivery vector as described herein.

According to another aspect, the present disclosure provides an *ex vivo* method of providing a linear single stranded donor template to a cell for genome editing, comprising the use of a delivery vector as described herein, preferably wherein said linear single stranded nucleic acid is a donor template.

According to another aspect, the present disclosure provides an *ex vivo* method as described herein wherein said nuclease is a guided nuclease, optionally Cas9 or a variant thereof.

With any of the methods described herein, the delivery vector of the ~~invention~~disclosure is introduced into a cell. The delivery vector can be transfected by any suitable means, including chemical, physical or viral.

Detailed Description of the Invention

The construct of the invention is a closed circular polynucleotide comprised of a single strand. The sequence of said construct is designed such that it can be apportioned into at least three sections, although the boundaries of these sections may be adaptable, varying depending on the cleavage site of the nuclease. The construct is preferably a delivery vector, intended for the delivery of a linear single stranded nucleic acid. The first section and third sections include some complementarity, such that under appropriate or physiological conditions, these sections are capable of base-pairing and forming a duplex or stem structure. This duplex is a result of self-complementary sequences within the polynucleotide. The second section intervenes between the first and third sections, and includes the sequence for the linear single stranded nucleic acid for delivery. This second section, since it is positioned between the two complementary sequences, will generally form a “loop” of nucleic acid, which starts and ends at the first and third sections which are duplexed. It will generally be

represented as a single strand, since no regions of complementarity with other sequences within the polynucleotide will intentionally be included in order for the delivery vector to work as intended. However, single stranded nucleic acids are well known for assuming some secondary structure, and therefore this second section may be in any possible conformation, including one or more of hairpins, loops, and pseudoknots, including sections of linear nucleic acid. The “loop” as used herein may also be considered to be the section of the polynucleotide that is not designed to form the duplex part of the delivery vector, but this does not preclude it from self-annealing. If homology arms are present, these may be capable of self-annealing into a further duplex. The loop is effectively the “single stranded” sequence for delivery, trapped by the duplex section which can be processed in order to release the single strand. Whilst the term “loop” is used, it is clear that larger loops (for example, 6 nucleotides and above) may have their own secondary structure.

Claims

1. A nucleic acid delivery vector comprising a circular single stranded polynucleotide said vector comprising:

(a) a duplex formed from a first section and a third section of said polynucleotide, said sections including sequences which are complementary;

(b) a loop formed from a second section, said section separating the first and third sections;

wherein said duplex includes a recognition sequence for a targeted nuclease.

2. The nucleic acid delivery vector of claim 1 wherein said vector delivers a linear single stranded nucleic acid, wherein said single stranded nucleic acid is present within the second section.

3. The nucleic acid delivery vector of claim 2 wherein the linear single stranded nucleic acid may be any one or more of: a nucleic acid enzyme, an aptamer, a donor template, an mRNA, a functional RNA, or an antisense nucleic acid.

4. The nucleic acid delivery vector of claim 2 or 3 wherein the linear single stranded nucleic acid has a free 5' and 3' end once released from the delivery vector.

5. The nucleic acid delivery vector of claim 1 wherein said vector is a closed circular polynucleotide, optionally a closed DNA or closed RNA.

6. The nucleic acid delivery vector of any previous claim wherein said nuclease is a guided nuclease, optionally a nuclease associated with gene editing, preferably Cas9.

7. The nucleic acid delivery vector of any one of claims 1 to 5 wherein the nuclease binds to the recognition sequence without a guide.

8. The nucleic acid delivery vector of any previous claim wherein said vector is for use in therapy ~~a cell~~.

9. An *ex vivo* method of providing a linear single stranded nucleic acid to a cell, comprising the use of a delivery vector as claimed in any one of claims 1 to 8.

10. An *ex vivo* method of providing a linear single stranded donor template to a cell for genome editing, comprising the use of a delivery vector as described in any one of claims 1 to 8, preferably wherein said linear single stranded nucleic acid is a donor template.

11. An *ex vivo* method as claimed in claim 10 wherein said nuclease is a guided nuclease, optionally Cas9 or a variant thereof.