Cleaving Double-Stranded DNA at User-Chosen Sites

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The Wisconsin Alumni Research Foundation (WARF) is seeking commercial partners interested in developing a method of using the Ref nuclease derived from the bacteriophage P1, in combination with a RecA protein-bound targeting oligonucleotide, to cleave DNA at any desired target sequence.

Overview

Restriction enzymes are used widely to cleave double-stranded DNA. However, a given restriction enzyme can only cleave a DNA molecule at the specific nucleotide sequence that corresponds to the recognition site of the enzyme. Because the number of available restriction enzymes is limited, there are only a limited number of recognition sites at which double-stranded DNA can be cleaved. Additionally, because the recognition sites are generally short and may appear frequently in a DNA molecule, restriction enzymes often cleave DNA at more than one location. A new method of cleaving double-stranded DNA is needed.

The bacterial RecA protein is involved in homologous DNA recombination and DNA repair. RecA forms a filament on single-stranded DNA and then catalyzes strand exchange with double-stranded DNA. Ref (recombinant enhancement function) was identified in 1986 as a bacteriophage P1 protein that stimulated homologous recombination in a RecA-dependent manner.

The Invention

UW–Madison researchers have developed a method of using Ref to cleave double-stranded DNA at any desired target sequence. The researchers determined that Ref is a novel HNH class and RecA-dependent endonuclease. They have shown that Ref, in combination with RecA and a single-stranded DNA targeting oligonucleotide, can specifically cause cleavage of double-stranded DNA at a site complementary to the oligonucleotide.

Applications

- Cleaving double-stranded DNA at user-chosen sites
- Potentially useful to create gene knockouts in eukaryotic or bacterial cells for use in research
- Gene therapy

Key Benefits

- Provides a means of cleaving double-stranded DNA at user-chosen sites rather than depending on cleavage at the recognition sites of conventional endonucleases
- Because the target sequence is much longer than a restriction enzyme recognition site, the DNA molecule likely is only cleaved at

For More Information About the Inventors

- Michael Cox
- James Keck

Publications

- Gruenig M.C., Lu D., Won S.J., Dulberger C.L., Manlick A.J., Keck J.L. & Cox M.M. 2011. Creating Directed Double-Strand Breaks with the Ref Protein: A Novel Reca-Dependent Nuclease from Bacteriophage P1. J. Biol. Chem. 286, 8240-8251. [Epub Dec. 30, 2010]

Tech Fields

- Research Tools : DNA & RNA tools

For current licensing status, please contact Jennifer Gottwald at jennifer@warf.org or 608-960-9854