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The principle and application of restriction enzyme in genetic engineering

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ABSTRACT

Restriction enzymes also known as restriction endonucleases (REases) are the basic tools of molecular biology. They are enzymes that cleave double-stranded deoxyribonucleic acids (DNAs) in a sequence-specific manner and are ubiquitously present among prokaryotic organisms. They form part of the restriction-modification systems, which usually consist of an endonuclease and a methyltransferase. Restriction endonucleases have been the workhorse of molecular biology for the past 30 years. They catalyze the breakage of phosphodiester bonds on DNA backbones at specific sites and, together with their companion methyltransferases, are part of bacterial defense systems against the invasion of bacteriophages. Their working principle as well as their applications since their discovery has greatly impacted and enhanced genetic engineering which this review article hopes to elaborate.

INTRODUCTION

Restriction endonucleases are bacterial enzymes that cleave DNA at specific sites. The resulting DNA fragments may be separated electrophoretically in gel to form specific restriction patterns [1-8]. These enzymes occur naturally in bacteria as a chemical weapon against invading viruses and cut both strands of DNA when certain foreign nucleotides are introduced into the cell [9].

RestrictionModification (R-M) enzymes are classified into three main groups designated types IIII; based on their subunit structure, cofactor requirements, sequence recognition and cleavage position [10,11,4]. Type II systems are the most well known, having found widespread use in molecular cloning. They comprise separate methylases and homodimeric restriction endonucleases which recognize short, usually palindromic sequences of 4-8 basepairs (bp) in the presence of Mg^{2+} and cleave the DNA within or in close proximity to the recognition sequence [12,13]. Cleavage is catalytic and typically occurs either within, or immediately adjacent to the palindromic sequence [14].

Type III RM enzymes are tetrameric holoenzymes that possess sequence-specific methylation, restriction and DNA dependent nucleoside triphosphatase activities [11]. The sequence recognized is 56 bp in length and cleavage typically occurs 2527 bp away from, and to one side of the recognition sequence [13].

The most complex of the group are the type I enzymes which were the first R-M systems discovered. Type I restriction endonucleases (REs) consist of three different subunits: methylase (M), restriction (R) and specificity (S) encoded by the *hsdM*, *-R* and *-S* genes, where hsd refers to Host Specificity for DNA (15). Together, they form an intriguing, multifunctional complex which can either restrict or modify DNA. Here, the mode of action of the complex is dictated by the methylation state of the recognition sequence [16]. A fully methylated site results in no action being taken and in enzyme dissociation; hemi-methylated target sequences direct the enzyme into a protective methylation mode producing fully methylated DNA, while unmethylated DNA shifts the enzyme into a destructive (and protective) restriction mode. It is in this protective mode that type I enzymes restrict foreign DNA and thereby maintain the integrity of the host genome [13].

PRINCIPLES

Restriction endonucleases degrade foreign DNA upon its introduction into a cell. These enzymes recognize particular base sequences, called *recognition sequences* or *recognition sites,* in their target DNA and cleave that DNA at defined positions [17]. Once inside a bacterial host, the restriction enzymes selectively cut up foreign DNA in a process called restriction [18]. To cut the DNA, a restriction enzyme makes two incisions, once through each sugar-phosphate backbone (i.e. each strand) of the DNA double helix. The restriction endonucleases catalyze the breakage of the phosphodiester bonds on DNA backbones at specific sites and, together with their companion methyl transferases, are part of bacterial defense systems against the invasion of bacteriophages [7]. The most well studied class of restriction

enzymes comprises of the so-called type II restriction enzymes, which cleave DNA *within* their recognition sequences. Other types of restriction enzymes cleave DNA at positions somewhat distant from their recognition sites [17]. Type I restriction endonucleases which are intriguing, multifunctional complexes that restrict DNA randomly, do so at sites distant from the target sequence. Restriction at distant sites is facilitated by adenosine triphosphate (ATP) hydrolysis-dependent, translocation of double-stranded DNA towards the stationary enzyme bound at the recognition sequence. Following restriction, the enzymes are thought to remain associated with the DNA at the target site, hydrolyzing copious amounts of ATP [13].

To restrict DNA, the REase complexes first recognize and bind to specific, non-methylated, bi-partite and asymmetric DNA sequences embedded within double-stranded DNA (dsDNA) [19]). These sequences consist of two specific domains (35 bp in length) split by a non-specific spacer of defined length (68 bp) (20). Once bound to the target sequence, the enzyme hydrolyzes ATP with a $k_{cat}$of~100 000 min^{-1}, while simultaneously translocating dsDNA bidirectionally toward the holoenzyme anchored at the recognition sequence [21,19,22]. When translocation is impeded, typically thousands of base pairs distant from the recognition sequence, restriction occurs [19,2325]. Surprisingly, these enzymes are thought to remain bound to the nascent cleaved DNA where they continue to rapidly hydrolyze ATP for several hours [21,26-28]. That is, each holoenzyme is able to cleave only a single DNA substrate [13].

The orthodox type II enzymes are homodimers which recognize palindromic sites. Depending on particular features subtypes are classified but all structures of restriction enzymes show a common structural core comprising four β-strands and one α-helix. Like other DNA binding proteins, restriction enzymes are capable of non-specific DNA binding, which is the prerequisite for efficient target site location by facilitated diffusion. Non-specific binding usually does not involve interactions with the bases but only with the DNA backbone. In contrast, specific binding is characterized by an intimate interplay between direct (interaction with the bases) and indirect (interaction with the backbone) readout.

Typically ~1520 hydrogen bonds are formed between a dimeric restriction enzyme and the bases of the recognition sequence, in addition to numerous van der Waals contacts to the bases and hydrogen bonds to the backbone, which may also be water mediated. The recognition process triggers large conformational changes of the enzyme and the DNA, which lead to the activation of the catalytic centres. In many restriction enzymes the catalytic centres, one in each subunit, are represented by the PD . . . D/EXK motif, in which the two carboxylates are responsible for Mg^{2+} binding, the essential cofactor for the great majority of enzymes. Cleavage in the two strands usually occurs in a concerted fashion and leads to inversion of configuration at the phosphorus. The products of the reaction are DNA fragments with a 3′-OH and a 5′-phosphate [12].

Applications

The first experiments demonstrating the utility of restriction enzymes were carried out by Danna and Nathans and reported in 1971. This pioneering study set the stage for the modern practice of molecular biology in which restriction enzymes are ubiquitous tools, although they are often taken for granted. Another important use for restriction enzyme fragments was in the early days of DNA sequencing **[1].** In forensic applications, certain DNA substrates are cleaved by REase to generate a unique fingerprint [6].

Isolated restriction enzymes are used to manipulate DNA for different scientific applications. They are used to assist insertion of genes into plasmid vectors during gene cloning and protein expression experiments. For optimal use, plasmids that are commonly used for gene cloning are modified to include a short *polylinker* sequence (called the multiple cloning site, or MCS) rich in restriction enzyme recognition sequences. This allows flexibility when inserting gene fragments into the plasmid vector; restriction sites contained naturally within genes influence the choice of endonuclease for digesting the DNA since it is necessary to avoid restriction of wanted DNA while intentionally cutting the ends of the DNA. To clone a gene fragment into a vector, both plasmid DNA and gene insert are typically cut with the same restriction enzymes, and then glued together with the assistance of an enzyme known as a DNA ligase [29].

Restriction enzymes can also be used to distinguish gene alleles by specifically recognizing single base changes in DNA known as single nucleotide polymorphisms (SNPs) [30,31]. This is only possible if a SNP alters the restriction site present in the allele. In this method, the restriction enzyme can be used to genotype a DNA sample without the need for expensive gene sequencing. The sample is first digested with the restriction enzyme to generate DNA fragments, and then the different sized fragments separated by gel electrophoresis. In general, alleles with correct restriction sites will generate two visible bands of DNA on the gel, and those with altered restriction sites will not be cut and will generate only a single band. The number of bands reveals the sample subject′s genotype, an example of restriction mapping.

In a similar manner, restriction enzymes are used to digest genomic DNA for gene analysis by Southern blot. This technique allows researchers to identify how many copies (or paralogues) of a gene are present in the genome of one individual, or how many gene mutations (polymorphisms) have occurred within a population. This is also called restriction fragment length polymorphism (RFLP) [17].

CONCLUSION

Restriction enzymes since their discovery have proved to be very valuable to genetic engineering and molecular biology as a whole. They have offered unparalleled opportunities for new discoveries in research development. Though there have been a few challenges, further research will bring about the discovery of more of these enzymes with exceptional characteristics to further advance science and technology.

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