Topic Introduction

Restriction Enzymes

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Restriction enzymes provided the foundation on which molecular cloning was built, and they remain as essential tools in current recombinant DNA technology. The three classes of restriction enzymes and their features are introduced here.

THE DISCOVERY OF RESTRICTION ENZYMES

The abundance of restriction enzymes nowadays available results from a phenomenon first described in the 1950s. Four separate laboratories almost simultaneously discovered the phenomenon known as host-controlled modification, in which some bacteriophages grew well in some strains of *E. coli* and poorly in other, closely related strains (for review, see Arber 1965). Bacteriophages that grew poorly in a host-specific manner were said to show a restricted host range.

Using the replicative form of bacteriophage fd DNA, Arber and Linn (1969) were able to show that restrictive hosts contained an enzyme that degraded viral DNA and that the bacteriophage DNA was protected from degradation if it had previously been modified by methylation (Kuhnlein et al. 1969). In thinking about these two opposing components of the bacterial defense system, Arber presciently suggested that there were specific sites in the infecting bacteriophage genome that were targets for restriction and that methylation at these sites would be protective. In his 1965 review, he wrote:

The system of host-controlled modification and restriction provides a unique defense mechanism for the bacterial cell: DNA foreign to the strain is specifically identified and inactivated. This system is particularly effective toward a virus which has been recently introduced from a foreign environment, a virus to which the bacterial population has not as yet built up an immunity.

Arber and Linn's work stimulated others to search for and characterize additional restriction enzymes. Hamilton Smith and Kent Wilcox (1970) purified and characterized a cognate pair of restriction and modification enzymes from *Hemophilus influenzae*, a small Gram-negative coccobacillus. Dan Nathans and his coworkers then showed that this restriction enzyme could cleave SV40 DNA into specific, electrophoretically separable fragments that could be mapped onto the viral genome (Danna et al. 1973). The beauty of restriction/modification systems was captured in a piece written by 10-yr-old Sylvia Arber in 1978, the year that her father Werner Arber, together with Hamilton Smith and Dan Nathans, was awarded the Nobel prize:

When I come to the laboratory of my father, I usually see some plates lying on the tables. These plates contain colonies of bacteria. These colonies remind me of a city with many inhabitants. In each bacterium there is a king. He is very long, but skinny. The king has many servants. These are thick and short, almost like balls. My father

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calls the king DNA, and the servants enzymes. The king is like a book, in which everything is noted on the work to be done by the servants. For us human beings these instructions of the king are a mystery.

My father has discovered a servant who serves as a pair of scissors. If a foreign king invades a bacterium, this servant can cut him in small fragments, but he does not do any harm to his own king. Clever people use the servant with the scissors to find out the secrets of the kings. To do so, they collect many servants with scissors and put them onto a king, so that the king is cut into pieces. With the resulting little pieces it is much easier to investigate the secrets. For this reason my father received the Nobel Prize for the discovery of the servant with the scissors. (Published in a News and Views article in *Nature Structural Biology* [Conforti 2000].)

CLASSES OF RESTRICTION ENZYMES

Restriction enzymes are classified into types I, II, and III according to their domain structures, cofactor requirements, length and symmetry of recognition sequences, and position of cleavage. From a cloning point of view, the most important restriction enzymes belong to class II. Hundreds of class II enzymes are available commercially for use in almost every facet of molecular cloning. The ubiquity and variety of type II enzymes in different bacterial species is thought to result from a common evolutionary need for a strong defense mechanism against bacteriophage genomes and other invading DNAs.

Restriction enzymes of all three types have cognate DNA methylases that recognize the same DNA sequence as the endonuclease and catalyze the transfer of a methyl group to a specific base on each DNA strand within the recognition sequence. Methylation of restriction sites generally involves cytosine residues and generates 4-methylcytosine, 5-methylcytosine, or 5-hydroxymethylcytosine. Methylation can also occur on adenine residues, generating 6-methyladenine. Methylated recognition sites are protected from digestion by the cognate restriction enzyme.

Bacterial genes encoding a particular restriction enzyme and its companion methylase are adjacent to one another on the bacterial chromosome but are not necessarily in the same orientation. Tight physical linkage decreases the chance of the two genes being separated by recombination during bacterial conjugation—obviously a disastrous possibility.

The classes of restriction enzymes and their features, described in detail below, are summarized in Table 1.

Type I restriction enzymes recognize DNA sequences that are asymmetric and composed of two portions—one of 3–4 nucleotides, the other of 4–5 nucleotides, separated by a spacer of ~6–8 nucleotides. Type I enzymes do not cleave at their recognition sequences. Instead they cut unprotected

TABLE 1. Summary: Major classes of restriction enzymes

Type of enzyme	Structure	Cofactor in vitro	DNA recognition sequence	Cleavage site
I	Complex, with three different subunits (endonuclease [hsdR], methyltransferase [hsdM], recognition [hsdS])	SAM, ATP	Asymmetric and complex	>1000 bp from recognition site. The best-studied class I enzyme (LlaG1) cleaves at two asymmetric recognition sites in a head-to-head inverted repeat. The enzyme. The helicase domain of the enzyme catalyzes one-dimensional stepwise translocation of dsDNA between the recognition sites (Smith et al. 2009a,b). For review, see Bourniquel and Bickle (2002).
II	Homodimers. Endonuclease and methylase activities are on different molecules	Mg ²⁺	Dyad symmetry	At recognition site
III	Complex with 2 subunits (endonuclease and methylase recognition)	ATP, Mg ²⁺ , SAM (in some cases)		Type III restriction enzymes cleave DNA by long-range interaction between recognition sites arranged in a head-to-head or tail-to-tail configuration (van Aelst et al. 2010). Motion along the DNA is thought to occur by DNA sliding (Ramanathan et al. 2009; Szczelkun et al. 2010).

DNA at sites at least 1 kb away (for review, see Murray 2000). Although they are of no use to molecular cloners, type I restriction enzymes are of interest to biochemists because of their multisubunit structure and their ability to function as molecular motors (Bourniquel and Bickle 2002; Seidel et al. 2004; Smith et al. 2009a,b). They have strong functional relationships to recombination enzymes such as RecBC and to chromatin remodeling factors in eukaryotes.

Type II

Type II restriction enzymes are a heterogeneous collection of enzymes with a wide range of properties. The most common type II enzymes (e.g., HindIII, EcoRI) are components of binary modification/restriction systems consisting of a restriction endonuclease that cleaves a specific sequence of nucleotides and a separate methylase that modifies the same recognition sequence. An increasingly large number of restriction endonucleases of this type have been isolated and characterized, a great many of which are useful for cloning and other molecular manipulations.

The vast majority of type II restriction endonucleases bind to DNA as homodimers that recognize specific sequences that are 4, 5, or 6 nucleotides in length and display twofold symmetry. A few type II enzymes, however, bind as heterodimers that recognize longer sequences or sequences that are degenerate. The location of cleavage sites within the axis of dyad symmetry differs from enzyme to enzyme: Some cleave both strands exactly at the axis of symmetry, generating fragments of DNA that carry blunt ends, whereas others cleave each strand at similar locations on opposite sides of the axis of symmetry, creating fragments of DNA that carry protruding single-stranded termini. Cleavage by type II restriction enzymes yields a 5'-phosphate residue on one side of the cut and a 3'-hydroxyl residue on the other.

Comparison of the X-ray crystallographic structures of a number of type II enzymes shows that their active sites have diverged significantly and in many cases have evolved different mechanisms for DNA binding and cleavage. For example, BamHI and BglII recognize 6-base targets with the four central bases [^{5'}GATC^{3'}] in common. Surprisingly the two enzymes use completely different arrangements of amino acid side-chains to make contact with these central 4 base pairs (Lukacs et al. 2000). Galburt and Stoddard (2000) point out that the global differences in structure between the active sites of the two restriction enzymes suggest that their specificities could not easily be changed by point mutation. This tight structural specificity makes evolutionary sense: changing or slackening the cleavage specificity of a class II enzyme by simple amino acid substitution would have lethal consequences because the cognate methyltransferase would be unable to protect the new targets.

A few Type II enzymes display different properties. Type II B restriction enzymes, for example, contain more than one subunit, recognize discontinuous sequences (e.g., ^{5'}CGANNNNNNTGC^{3'}) require S-adenosyl methionine and Mg²⁺, and cleave on both sides of their recognition sites, releasing a small fragment of DNA. Type II M restriction endonucleases, such as DpnI, recognize and cleave only methylated DNA; DpnI requires methylation of the adenine residue in its target sequence (GATC).

Type III

Prototypical members of this group (EcoPI, EcoP15I, and PstII) recognize short asymmetric DNA sequences and cleave the DNA nonspecifically 25–28 nucleotides 3' to the recognition site (for review, see Bourniquel and Bickle 2002). Type III enzymes translocate DNA in a motoring reaction driven by hydrolysis of ATP (Meisel et al. 1995).

ONLINE DATABASES

REBASE is a comprehensive database of restriction enzymes and methylases of all types and is available at <http://rebase.neb.com/rebase/rebase.html>. REBASE is available in numerous formats in-

cluding an ASCII text file arranged in fields for easy formatting and for use with specific software programs for DNA analysis.

Wikipedia also maintains a database of restriction enzymes at http://en.wikipedia.org/wiki/List_of_restriction_enzyme_cutting_sites.

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