RESTRICTION ENZYMES

Like the immune systems of vertebrate eukaryotes, the restriction enzymes of bacteria combat foreign substances. In particular, restriction enzymes render the DNA of, say, an invading bacteriophage harmless by catalyzing its fragmentation, or, more precisely, by catalyzing the breaking of certain -O–P–O- bridges in the backbones of each DNA strand. The evolution of restriction enzymes helped many species of bacteria to survive; their discovery by humans helped precipitate the recombinant-DNA revolution.

Three types of restriction enzymes are known, but the term "restriction enzyme" refers here and elsewhere in this issue to type II restriction endonucleases, the only type commonly used in the study of DNA. (A nuclease is an enzyme that catalyzes the breaking of -O–P–O- bridges in a string of deoxyribonucleotides or ribonucleotides; an endonuclease catalyzes the breaking of internal rather than terminal -O–P–O- bridges.) Many restriction enzymes have been isolated; more than seventy are available commercially. Each somehow recognizes and binds to its own restriction sites, short stretches of double-stranded DNA with a specific base sequence. Having bound to one of its restriction sites, the enzyme catalyzes the breaking of one particular -O--P–O- bridge in each DNA strand.

The accompanying table lists a few of the more commonly used restriction enzymes and the organism in which each is found. The first three letters of the name of a restriction enzyme are an abbreviation for the species of the source organism and are therefore customarily italicized. The next letter(s) of the name designates the strain of the source organism, and the terminal Roman numeral denotes the order of its discovery in the source organism.

Restriction Enzyme	Source Organism	Base Sequence of Restriction Site
*Bam*HI	*Bacillus amyloliquefaciens*	5'-G|GATCC-3' 3'-CCTAG|G-5'
*Eco*RI	*Escherichia coli*	5'-G|AATTC-3' 3'-CTTAA|G-5'
*Hae*III	*Haemophilus aegyptius*	5'-GG|CC-3' 3'-CC|GG-5'
*Hin*dII	*Haemophilus influenzae*	5'-GT(C or T)|(A or G)AC-3' 3'-CA(G or A)|(T or C)TG-5'
*Mbo*I	*Moraxella bovis*	5'-|GATC-3' 3'-CTAG|-5'
*Not*I	*Nocardia otitidis*	5'-GC|GGCCGC 3'-CGCCGG|CG
*Taq*I	*Thermus aquaticus*	5'-T|CGA 3'-AGC|T

Also listed in the table are the base sequences of the restriction sites of the enzymes. The red line separates the ends of the resulting fragments. The restriction sites of many of the known restriction enzymes and of all the restriction enzymes listed in the table have palindromic base sequences. That is, the 5′-to-3′ base sequence of one strand is the same as the 5′-to-3′ base sequence of its complementary strand. Both the bridges broken by a restriction enzyme that recognizes a palindromic sequence lie within or at the ends of the sequence.

Note that most of the restriction enzymes in the table make "staggered" cuts; that is, they produce fragments with protruding single-stranded ends. Those "cohesive," or "sticky," ends are very useful. Suppose that a sample of human DNA and a sample of phage DNA are both fragmented with the same restriction enzyme, one that makes staggered cuts. When the resulting fragments are mixed, they will tend to hydrogen bond with each other because of the complementarity of their sticky ends. In particular, some human DNA fragments will hydrogen bond to some phage DNA fragments. And that bonding is the first step in the creation of a recombinant DNA molecule.

A final point about restriction enzymes is the problem of how the DNA of a bacterium avoids being chopped up by the friendly fire of the restriction enzyme(s) it produces. Evolution has solved that problem also. A bacterium that produces a type II restriction endonuclease produces in addition another enzyme that catalyzes the modification of restriction sites in its own DNA in a manner such that they cannot serve as binding sites for the restriction enzyme.