A comparison of palindrome and pseudopalindrome cutters

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Type II restriction endonucleases are very diverse in sequence, yet nearly all of them share the fold and the metal-dependent catalytic mechanism. Most are active as dimers that match the two-fold symmetry of their recognition sequences. Although thousands of restrictases have been described, fewer than 20 crystal structures are available. To date, mostly “palindrome-cutters” that recognize true palindromes and generate overhangs with an even number of bases have been studied in detail.

Based on these data, a few general rules have been deduced: (a) “phenotype” predicts “genotype”, (b) changes in the recognition sequence, which do not affect the cleavage pattern, require mutations, but no alterations in quaternary structure, (c) changes in the cleavage pattern require radically different dimerization modes.

Interestingly, recent data indicate that “pseudopalindrome-cutters” that recognize pseudopalindromic sequences and generate overhangs with an odd number of bases violate these rules, at least in some cases. Pseudopalindrome cutters are similar to palindrome cutters, especially in their dimerization modes, yet generate different cleavage patterns. We have selected the pseudopalindrome cutter Ecl18kI (CCNGG) and the related palindrome cutter NgoMIV (G/CCGGC) for a detailed comparison. A high resolution structure of NgoMIV with DNA has been reported previously [1]. We have now crystallized Ecl18kI with DNA and collected native data to 1.7 Å resolution at BW6, DESY. The structure was solved by the MAD method, using data for the selenomethionine variant and a bromide soak, which were also collected at BW6, DESY.

A comparison of the refined Ecl18kI-DNA complex with the NgoMIV-DNA complex is instructive. The two restriction enzymes use a conserved “recognition module” to interact with their target sequences as predicted. To accommodate the extra nucleotides (N) at the center, Ecl18kI flips them out from the DNA duplex. This flip and the accompanying DNA kink cause a register shift by 1 bp making the distances between scissile phosphates in the NgoMIV and Ecl18kI cocrystal structures nearly identical. Ecl18kI is the first example of a restriction endonuclease that flips nucleotides to achieve specificity for its recognition site [2].

References
