RATIONAL ALTERATION OF DNA RECOGNITION: SIMPLE MUTATIONS THAT CHANGE TYPE IIG RESTRICTION ENZYME SPECIFICITY

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Type II restriction endonucleases (REs) robustly recognize specific DNA sequences. Here we present a methodology that allows us to rationally engineer DNA recognition specificity in the MmeI family of REs[1]. First, alignments of the RE protein sequences and of their DNA recognition sequences are formed, then the two alignments are interrogated to identify correlations between the aligned amino acid residues and the DNA base pair present at each position in the aligned recognition sequences. From such correlations we have identified pairs of amino acids likely to specify DNA base recognition for five of the six variable positions in the RE recognition sequences. We then alter recognition specificity by mutating the amino acids at the identified positions to those correlated with recognition of the desired new base. To date we have successfully altered specificity at four positions in the recognition sequences. Individual enzymes tolerate changes at multiple positions; for example, NmeAIII has been altered at three positions, from GCCGAG21/19 to GCGRAC21/19. The majority of the enzymes so altered have specific activity similar to that of the wild type enzymes. Thus using simple and predictable mutagenesis we demonstrate that it is possible to create hundreds of unique new Type II RE specificities. These enzymes provide a unique scaffold to investigate the interaction of various amino acid residues with particular DNA base pairs, and opportunity to study the evolution of DNA specificity.

1. Morgan, R.D. and Luyten, Y.A. (2009) Rational engineering of type II restriction endonuclease DNA binding and cleavage specificity. *Nucleic Acids Res*, **37**, 5222-5233.