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Molecular Evolution of a TALE Protein to Change DNA Binding Manner

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Artificial DNA binding proteins binding to desired DNA sequences are useful to control transcription of various genes and/or gene editing. So, they can be powerful tools for synthetic biology. Transcription activator-like effectors (TALEs) are sequence-specific DNA binding proteins secreted by the bacterial pathogen *Xanthomonas*. Their DNA binding specificity is determined by a series of tandem repeats of typically 34 highly conserved amino acids, containing repeat variable di-residues (RVDs) at positions 12 and 13, that define the base preferences of a repeat. Because of the simple one-to-one base recognition by each repeat, TALEs can be readily designed to target desired DNA sequences just by replacing the RVDs. Though TALEs have the target sequence versatility determined by tandem repeats regions, almost all TALE binding sites are preceded by a highly conserved 5' terminal T nucleotide. N-terminal noncanonical repeat (-1st repeat) has been thought to interact specifically with the 5'-T. However, the details of the base recognition mode are still unclear. In this study, we substituted amino acids that correspond to the residues of the adjoining loops of the -1st repeat and verified the contributions to the 5'-T recognition. In addition, we performed directed evolution of the -1st repeat to bind to non 5'-T sequences using a bacterial 1-hybrid assay.

We introduced a point mutation in a tryptophan residue at the adjoining loop of the -1st repeat of dHax3. The DNA bindings of the mutated dHax3s were examined by luciferase reporter assays in HeLa cells. Only the substitutant with tyrosine showed a comparable activity to wild type. A significant decrease in the luciferase activity was observed when the tryptophan residue was substituted with each of the other 18 amino acids. In addition, all of the mutants showed a DNA binding preference to 5'-T. This result suggests that tryptophan and tyrosine play an important role in the DNA recognition by the -1st repeat, but the recognition of the 5'-T appears not to be so simple as the canonical repeat domains. Therefore, we randomized the four amino acid residues in the loop region and intended to change the recognition pattern of the 5'-terminal nucleotide. Through a bacterial one-hybrid screening using a reporter vector with a dHax3 binding site starting from 5'-C, we obtained highly conserved amino acid sequences of the loop region. The selected dHax3 mutant showed significantly strong DNA binding activity to the binding sites starting from non 5'-T. This result directly indicates the importance of the -1st repeat on the recognition of the 5'-terminal nucleotide.