DNA Binding Action of a TAL Effector Captured In 3D

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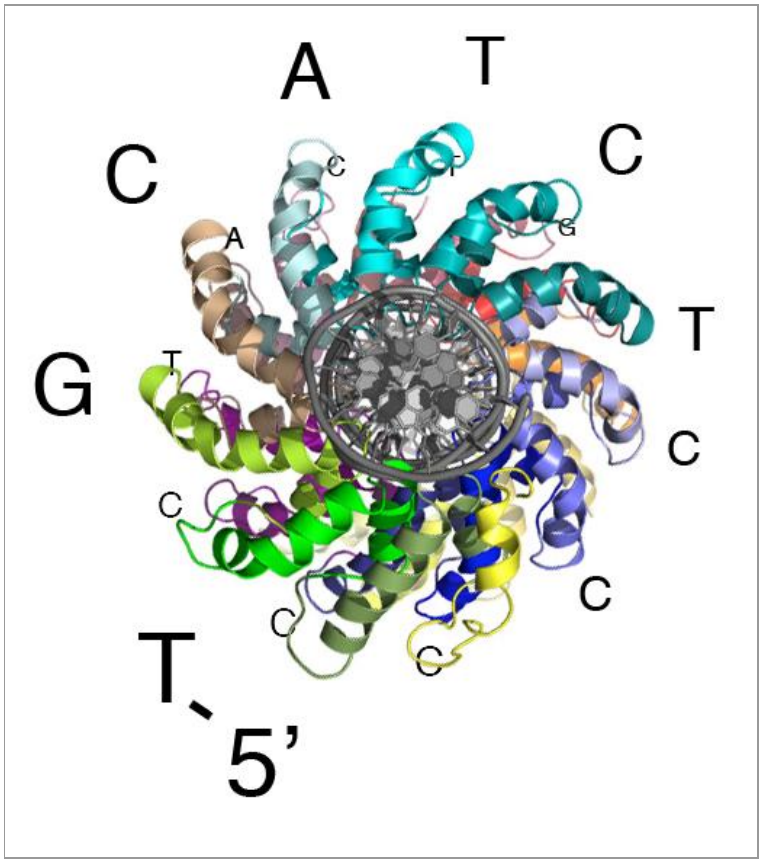
Nature is replete with DNA-binding proteins capable of gene-specific DNA recognition. When coupled to an active domain that cuts DNA, modifies transcription or initiates replication, such proteins can be shaped into tools. Among the most useful of these tools are arrays of zinc fingers fused to nucleases or transcriptional activators. Under optimal conditions, the resulting proteins allow for targeted mutagenesis or gene regulation, though it has been difficult to achieve high and customizable specificities using zinc finger arrays. Recently discovered proteins called transcription activator-like (TAL) effectors, which are produced naturally by certain bacterial pathogens of plants, promise to overcome these limitations. TAL effectors recognize DNA in a modular manner, allowing for greater target sequence specificity and flexibility. TAL effectors possess 13 to 28 central repeat motifs that underlie DNA specificity. Each repeat is typically 34 amino acids in length and contains a pair of residues at positions 12 and 13 called repeat-variable di-residues (RVDs). Different RVDs vary in their affinities for specific nucleotides.

Realizing the full biomedical potential of TAL effectors will require a better understanding of RVD-nucleotide preferences, effects of RVD composition on DNA affinity and the nature of the protein-DNA interface. Major progress toward these goals has been achieved by Dr. Amanda Mak and principal investigator Dr. Barry Stoddard (both in the Basic Sciences Division) in collaboration with Dr. Philip Bradley (Public Health Sciences Division) and others. Mak *et al.* successfully crystallized the central portion of the TAL effector PthXo1 bound to a 36 base pair fragment containing its target in the rice genome. The authors then solved the structure of the bound construct using X-ray crystallography in combination with high-throughput computational prediction, validating their best model by means of selenomethionyl derivitization. They found that the repeat motifs of PthXo1 self-associate to create a right-handed superhelix wrapped around the DNA major groove (see fig.). Individual repeats form similar left-handed two-helix bundles, with the RVD placed between these smaller helices and presented to the DNA. Mak and co-authors also demonstrated essential features underlying DNA interaction specificity for the most common repeat types, laying the necessary groundwork for developing TAL effectors into powerful agents of experimental genetics and targeted gene editing.

Mak AN-S, Bradley P, Cernadas RA, Bogdanove AJ, Stoddard BL. 2012. The crystal structure of TAL effector PthXo1 bound to its DNA target. *Science Express* online, 5 January 2012, doi:10.1126/science.1216211.



Modified from Mak et al. (2012)

Structure of the DNA-targeting domain of TAL effector PthXo1 bound to its target in the promoter of rice gene Os8N3 (viewed parallel to long axis of the DNA double strands, shown in gray). Part of the target DNA sequence is provided schematically outside the PthXo1 superhelix, even though the bound DNA is internal. PthXo1 contains 23.5 canonical repeat motifs (colored differently). These motifs possess different RVDs that bind preferentially with different nucleotides, here for example: NN with G (or A), HD with C, NI with A, HG with T, HD again with C, NG with T, etc. A 5' T is universally bound by TAL effectors via the protein sequence immediately preceding the first canonical repeat.