# TALEs: Unique Proteins for Programmable DNA Modification

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## Abstract

Transcription activator-like effectors (TALEs), which were originally isolated from a plant pathogen *Xanthomonas*, are high-affinity DNA binding proteins (Pennisi, 2012). These proteins have multiple 33 to 35-amino acid helix-loop-helix repeats, each with one variable amino acid pair in the loop that interacts with a specific nucleotide in the DNA helix. These repeat variable diresidues (RVDs) in the TALE protein make for a unique specificity, as there is a predictable pattern of correspondence between the RVD and the DNA base. The elucidation of this "code" allows the engineering of TALES that target specific genes. TALE fusion proteins offer the potential to target specific genes for DNA modification (Mak, 2012).

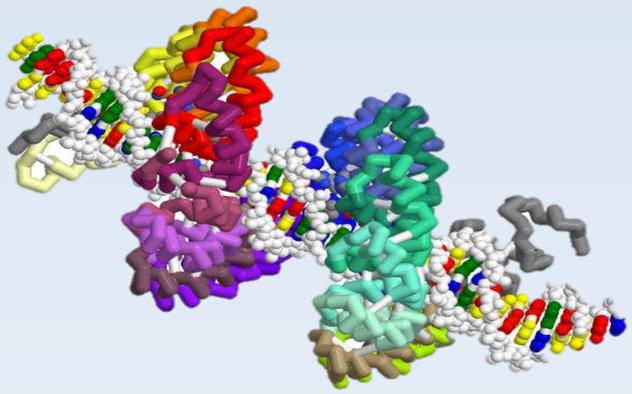


**Figure 1** TALE PhtXo1 bound to its target DNA. The image is a Jmol rendering from pdb file 3UGM colored to illustrate the 23.5 repeats.

## Introduction

Naturally occurring transcription activator-like effector proteins, or TALEs, are bacterial proteins from the genus *Xanthomonas*, which cause the infected plant to over express specific genes (Pennisi, 2012). TALE PhtXo1 contains 23.5 repeats, each with a 34 amino acid helix-loop-helix motif. The loop of each repeat contains a "repeat variable diresidue", or RVD, at positions 12 and 13 which will bind to a particular nucleotide on the DNA. TALE proteins can be fused with DNA-modifying enzymes, such as nucleases. After binding to the DNA the TALE, now known as a TALEN, will cause breaks in the DNA which will lead to knockout and knock-in organisms. Using these organisms we can study genome engineering and possible gene therapies (Mak, 2012).

## Methods

The PDB file used was 3UGM and the model was built by the Center for BioMolecular Modeling. The model was constructed to coordinate with the color scheme from the paper by Mak *et al*. (2012).

## TALE Architecture

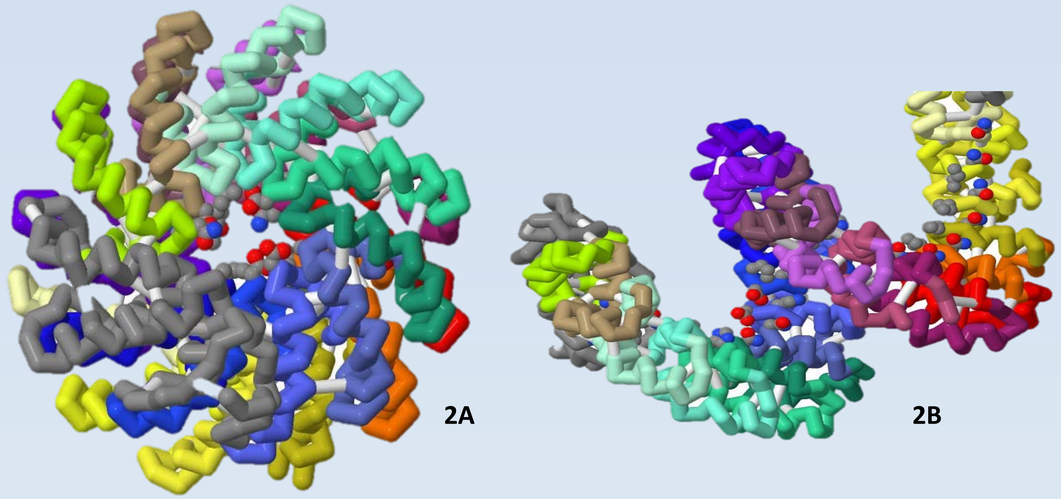



**Figure 2** TALE PhtXo1 without its target DNA. The individual repeats are represented by different colors, matching the schematic in Fig. 3A. Side chains of the RVDs are shown in space-fill 1.25, with CPK color scheme. (**2A)** Top view. (**2B)** Side view. The image is a Jmol rendering from pdb file 3UGM colored to illustrate the 23.5 repeats.

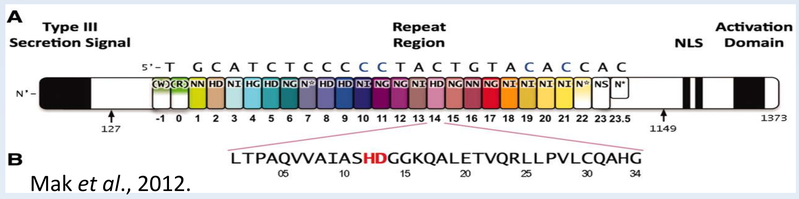
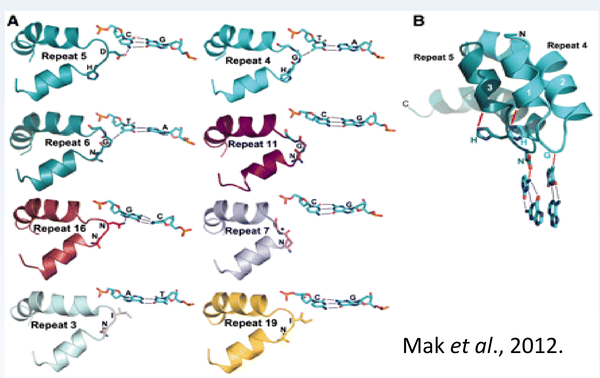



**Figure 3**
Organization of the PhtXo1 repeat region.
**(A)** Each of the 23.5 repeats (numbered under the schematic) is represented by a different color. Bases corresponding to each repeat are listed above the schematic. shows the 23.5 repeats, each represented by a different color. **(B)** The sequence of repeat number 14 is shown, with the RVD residues shown in red.




C

| RVD | DNA Base |
|---|---|
| HD | C |
| NG | T |
| NI | A |
| NN | G/A |
| NS | A/C/T/G |
| N* | C/T |
| HG | T |

**Figure 4**
**(A)** Selected repeats showing RVD 12 stabilizing the loop structure and RVD 13 interacting (or not interacting) with its corresponding DNA base. **(B)** Two adjacent repeats forming a tightly packed left handed bundle of helices. **(C)** 90% of all repeats have 7 RVDs shown in the table. (N* has a reduction in loop length by one residue)

## TALE Engineering

TALEs fused with nucleases (TALENs) can cleave DNA at points close to TALE binding, allowing for gene inactivation or DNA insertion in embryos leading to knockout and knock-in organisms, respectively. After the TALE binds to the DNA it becomes a transcription activator-like effector nuclease, and the DNA will be cleaved creating a double stranded break in the DNA. This DNA break will be repaired by homology directed repair, causing the DNA to contain errors. If a piece of foreign DNA is introduced it can be used as a template for DNA repair. TALEs can be fused with other enzymes to modify DNA at a specific place in the genome, in order to activate or inactivate a gene. An example would be the fusion of a TALE with a methyltransferase. Methyltransferases are responsible for DNA methylation to repress gene expression. TALEs can be engineered to recognize a certain DNA sequence and when fused with a methyltransferase, can target that specific sequence for methylation. There may be potential applications in cancer research and cancer therapy as some cancers have a mutation in the methyltransferase and therefore are unable to methylate the DNA and prevent mutated genes from being transcribed. Through the use of TALEs being fused with methyltransferases scientists can study the methylation of certain DNA sequences and how this affects different diseases. Current researchers are looking into targeted DNA methylation of a specific gene in human cells resulting in a large downregulation of the targeted gene expression (Siddique et al., 2012).

## Acknowledgements

We would like to thank Jasmine Reed for her earlier contributions to this project.

## References

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The CREST Program is funded by grant #1022793 from NSF-CCLI.