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**Title:** Characterization and DNA-Binding Specificities of Ralstonia TAL-Like Effectors

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**Abstract:** We report the characterization of three Ralstonia TAL-like effectors, which mediate DNA binding and can be used as customizable architectures for DNA targeting. We determined DNA-binding specificities of novel repeat variable di-residues (RVDs) and devised a repeat assembly approach for engineering Ralstonia solanacearum TALE-like proteins (RTLs). Transcription activator-like effectors (TALEs) from Xanthomonas sp. have been used as customizable DNA-binding modules for genome-engineering applications. Ralstonia solanacearum TALE-like proteins (RTLs) exhibit similar structural features to TALEs, including a central DNA-binding domain composed of 35 amino acid-long repeats. Here, we characterize the RTLs and show that they localize in the plant cell nucleus, mediate DNA binding, and might function as transcriptional activators. RTLs have a unique DNA-binding architecture and are enriched in repeat variable di-residues (RVDs), which determine repeat DNA-binding specificities. We determined the DNA-binding specificities for the RVD sequences ND, HN, NP, and NT. The RVD ND mediates highly specific interactions with C nucleotide, HN interacts specifically with A and G nucleotides, and NP binds to C, A, and G nucleotides. Moreover, we developed a highly efficient repeat assembly approach for engineering RTL effectors. Taken together, our data demonstrate that RTLs are unique DNA-targeting modules that are excellent alternatives to be tailored to bind to user-selected DNA sequences for targeted genomic and epigenomic modifications. These findings will facilitate research concerning RTL molecular biology and RTL roles in the pathogenicity of Ralstonia spp.

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