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TAL effectors from *Xanthomonas*: design of a programmable DNA-binding specificity

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Abstract

Xanthomonas spp. are Gram-negative bacteria with powerful molecular weapons to attack their plant hosts. Key for pathogenicity of *Xanthomonas* is a type III secretion system that injects a cocktail of effector proteins into plant cells to function as potent virulence factors. TAL (transcription activator-like) effectors from *Xanthomonas* function as transcriptional activators of plant genes in the plant nucleus. They contain a central domain of tandem, near-identical 34 amino-acid repeats. Each repeat recognizes a single base pair in a contiguous DNA sequence and two adjacent hypervariable amino acids per repeat specify the base that is bound. This modular DNA-binding code allows a simple reprogramming of DNA-binding specificity, a feature with high potential for biotechnology. We developed a method called "Golden TAL Technology" that allows a flexible assembly of TAL proteins with a designed order of repeats.

Key words: Transcription factor, AvrBs3, zinc-finger proteins, golden gate cloning, TALE.

Introduction

Pathogenicity of plant pathogenic *Xanthomonas* relies on the delivery of virulence proteins, so called effectors, into target plant cells. Transport of effectors is facilitated by a specialized type III secretion system (Hrp) which spans both bacterial membranes and employs a hollow exterior conduit (the Hrp-pilus) that traverses the plant cell wall and delivers effectors across the plant plasma membrane via a translocon (Büttner and Bonas, 2010).

Typically, *Xanthomonas* strains harbor approx. 30 different effectors. In most cases their molecular activities are still unknown. TAL (transcription activator-like) effectors form a large and important effector family that is nearly exclusively found in *Xanthomonas* (Boch and Bonas, 2010). They function as transcription factors of plant genes and several TAL effectors induce expression of sugar exporters (Chen *et al.*, 2010).

TAL effectors contain a central domain of tandem near-identical 34-amino acid repeats (2 to 34). Each repeat binds to one DNA base pair in a contiguous sequence. Specificity of the repeats is determined by two hypervariable amino acids per repeat. Rearranging the repeats yields novel and predictable DNA-binding specificities (Boch *et al.*, 2009; Moscou and Bogdanove, 2009). TAL-nuclease fusions have been used to specifically edit the human genome (Miller *et al.* 2010).

Materials and methods

Golden TAL Technology

Four key TAL repeat types (NI, HD, NN, NG; table 1) were chosen that specifically recognize the four bases of the DNA. Individual TAL repeats were cloned with flanking *BpiI* sites. One to six repeats are ligated into an assembly vector replacing a *lacZ* selection marker by *Bpi*I cut-ligation (step I). Several repeat assemblies are combined together with fragments encoding N- and C-terminus of the TAL effector, respectively, into a target expression vector by *Bsa*I cut-ligation (step II).

Cut-ligations were set up with 50-100 ng of each plasmid, 1 µl restriction enzyme (*Bpi*I or *Bsa*I), 2 µl ATP (10 mM), 2 µl restriction buffer no. 4 (NEB), 1 µl T4-ligase (5 u/µl) in a 20 µl reaction. Reactions were incubated for 1 h at 37°C followed by 20 min. at 70°C to inactivate the enzymes. For further details please see (Geißler *et al.*, 2011).

Table 1. DNA-specificity of individual TAL effector repeats.

TAL repeats[a]	DNA specificity[b]
NI	A
HD	C
NN	G/A
NK	G
IG	T
NG	T
HG	T

[a] According to amino acids 12 and 13 per repeat.
[b] Only upper strand base is shown.

Results

Construction of designer TAL effectors

TAL effector repeats are highly repetitive on protein as well as DNA level which complicates cloning approaches. Our initially constructed artificial TAL effectors with novel repeat arrangements were based on

a random assembly of individual modules of four key repeats encoding a specificity for the four DNA bases, respectively (table 1). This cloning was possible, because exactly one restriction site for the type IIs restriction enzyme *Esp3*I is naturally present in each TAL repeat. Type IIs restriction enzymes have separate recognition and restriction sites, which lead to non-palindromic overhangs and a linear unidirectional ligation of TAL repeat fragments (Boch *et al.*, 2009).

To construct TAL effectors with a given order of repeats and, therefore, a programmed DNA-specificity, it was essential to align TAL effector repeats in a more controlled fashion. We improved our initial construction method and modified a method termed golden gate cloning (Engler *et al.*, 2008). In essence, golden gate cloning describes the assembly of a series of fragments containing specific overhangs that have been generated with flanking type IIs restriction enzymes. The fragments are designed such that correct ligation products lack the applied restriction site and are enriched in the reaction.

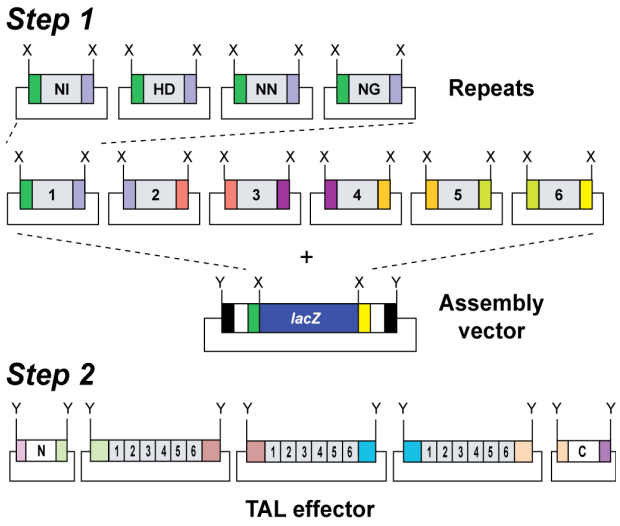

Figure 1. The golden TAL technology. TAL effectors with a designed repeat composition were assembled in two cut-ligation steps. X, *Bpi*I; Y, *Bsa*I. Same colors indicate matching overhangs.
(In colour at www.bulletinofinsectology.org)

TAL proteins with a desired order of repeats were constructed using a two-step assembly technique. First, a library of four key repeats with specific flanking type IIs restriction sites was constructed. Up to six repeats were combined into an assembly vector (figure 1). Second, several assembly vectors were combined with fragments encoding N- and C-terminal regions of TAL effectors into an expression vector (Geißler *et al.*, 2011).

Our setup allows a very flexible generation of TAL effectors with any designed order of repeats. Generation of programmable DNA-binding specificities is now easily possible.

Discussion

The controlled assembly of TAL effectors with a designed repeat composition is key to use these proteins as programmable DNA-targeting devices. Our golden TAL technology allows an easy assembly of TAL effectors with designed repeats. In addition, it is flexible to incorporate N- and C-terminal variations, as well as different tags as modules in the cut-ligation reaction. This should greatly facilitate the adoption of this technique also for the generation of fusion proteins (e.g. TAL-nucleases) for biotechnology.

Several groups have recently developed similar techniques. The tools that are currently generated will enable a broad applicability of the TAL technology for different biological fields.

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