The TALE-ChAP: a novel *in planta* method to identify proteins that associate with a promoter of choice

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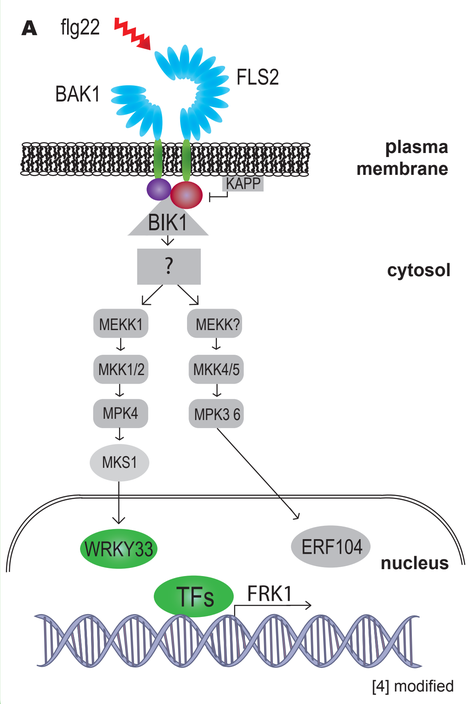
Introduction

To identify regulating proteins at promoters we want to establish a new *in vivo* method. The basis of this method is Chromatin Affinity Purification (ChAP), that we will combine with the Transcription Activator Like Effectors (TALEs) technology (TALE-ChAP). The TALE repeats can be modified to target a DNA sequence of choice. We will use designer TALEs (dTALEs) to target different motifs in *pFRK1* after flg22 signaling. The dTALEs will be used to purify DNA-protein complexes. The proteins that are bound to the DNA near the dTALE-binding sites will be identified by mass spectrometry (MS).

Plants recognize pathogens by highly conserved structures, called molecular associated patterns (MAMPs) like the bacterial peptide flagellin22 (flg22). In *A. thaliana* flg22 is perceived by the receptor Flagellin Sensitive 2 (FLS2) (Fig 1) [1,4]. The signal is translocated via MAPK cascades into the nucleus where transcription factors (TFs) modulate expression of pathogen responsive genes. The *Flg22-induced Receptor-Like Kinase1* (*FRK1*) is such a pathogen responsive gene that is induced after flg22 signaling [2]. The TFs that bind in response to flg22 to the *FRK1* promotor (*pFRK1*) are not identified yet.

1 Perception of Flagellin22 in *Arabidopsis thaliana*



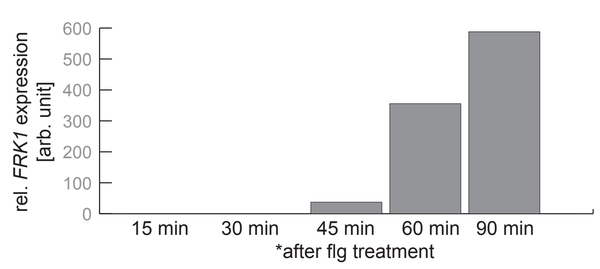
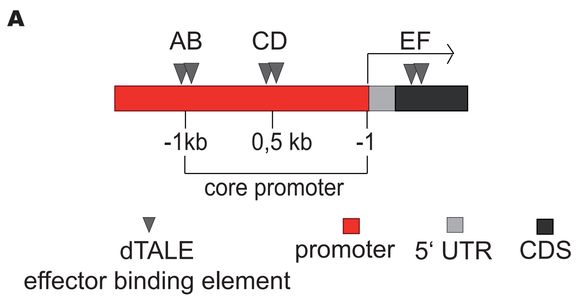
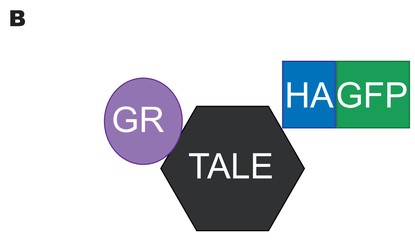


Figure 1:
A In *A. thaliana* the flg22 signal is perceived via the receptor FLS2 and translocated via MAPK cascades into the nucleus [1,4]. So far uknown TFs activate the expression of the defense responsive gene *FRK1*. WRKYs might play a role in the activation of *FRK1* expression because 9 W-boxes (TTGACY) can be found in *pFRK1* [3].
B Relative *FRK1* expression after flg treatment. Adult *A. thaliana* where infiltrated with flg22 or the inactive flg15Δ7 as negative control. The flg22 values where normalized to the flg15Δ7 values; *UBQ10* was used as reference gene.

2 The TALE-Chromatin Affinity Purification





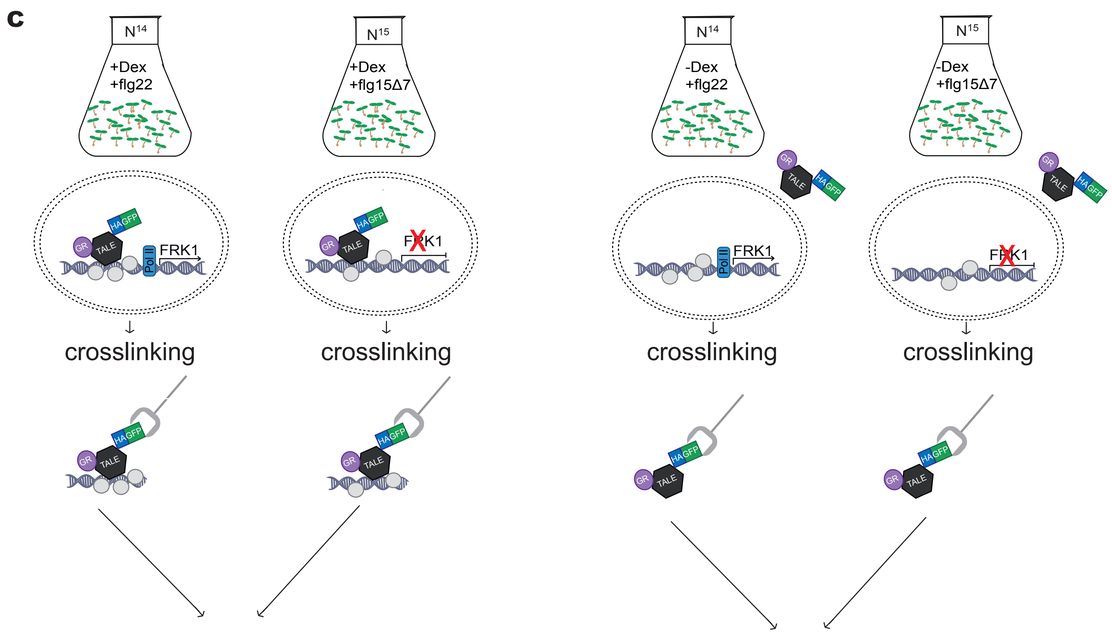
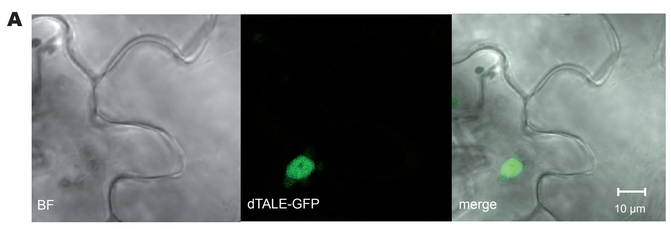
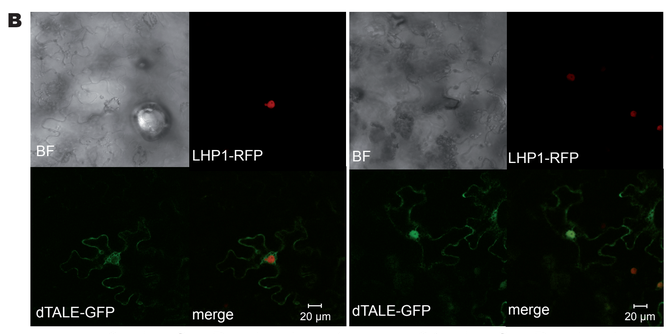


Figure 2:
A Schematic representation of *pFRK1*: The 6 dTALE effector binding elements (EBEs) (A-F) are indicated with triangles.
B Schematic representation of the *pFRK1* specific dTALE constructs: The GR-receptor enables Dexamethason (Dex) dependent nuclear translocation; the repeat region of the dTALEs mediate binding to the EBEs in *pFRK1*; the HA- and the GFP-Tag can be used for intracellular localization and Chromatin Affinity Purification (ChAP).
C Workflow of the TALE-ChAP: Stable dTALE expressing *A. thaliana* seedlings will be treated with flg22 to induce *pFRK1*. After Dex treatment the dTALEs will translocate to the nucleus where they bind to *pFRK1* and will be used as anchor proteins for the ChAP. The proteins that bound to *pFRK1* near the dTALE target sites will be analyzed via MS. Inactive flg15Δ7 and samples that where not Dex treated will function as negative controls.

3 Expression of dTALEs in *A. thaliana* and *N. benthamiana*





C

Background	dTALE	plants	cytosolic GFP signal (in nucleus after Dex treatment)
Col-0	A	17	10 (4)
	B	15	7 (1)
	C	4	3 (0)
	D	12	6 (2)
	E	1	1 (1)
	F	0	0
fls2	A	7	5 (2)
	B	13	4 (2)
	C	13	8 (8)
	D	15	3 (3)
	E	0	0
	F	0	0

Figure 3:
A Col-0 dTALE C line 4 90 min after Dex treatment.
B Translocation of transient expressed dTALE A into the nucleus in response to Dex treatment in *N. benthamiana.* 6 min & 81 min after Dex treatment (10 µM)
C Overview of the stable dTALE expressing *A. thaliana* lines. The 6 dTALE versions where transformed in the Col-0 and the flg22 insensitive *fls2* background.

Summary

We were able to show:
- Activation of *FRK1* expresssion after flg22 treatment within 45 min
- dTALE expression *in vivo* via fluorescence microscopy in transgenic *A. thaliana* lines.
- Dex dependent nuclear translocation of dTALEs in transiently transformed *N. benthamiana* leaves.

Outlook

With the TALE-ChAP we try to get an idea of flg22 induced protein dynamics at *pFRK1*.
Once the TALE-ChAP is established the principle can be transfered to any other promoter, independent of the organism.
The next experiments will include:
- Test the *in vitro* binding specificity of the dTALEs in a DPI-ELISA
- Test the *in vivo* binding specificity of the dTALEs in a promoter-reporter assay in tobacco leaves
- Verify that *pFRK1* is precipitated in a XChIP

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References

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Robatzek, S. and I.E. Somssich, Genes Dev, 2002. 16(9): p. 1139-49 **[3]**
Park, C.J., D.F. Caddell, and P.C. Ronald, Front Plant Sci, 2012. 3: p. 177 **[4]**