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Breaking the Code of DNA Binding Specifity of TAL-Type III Effectors

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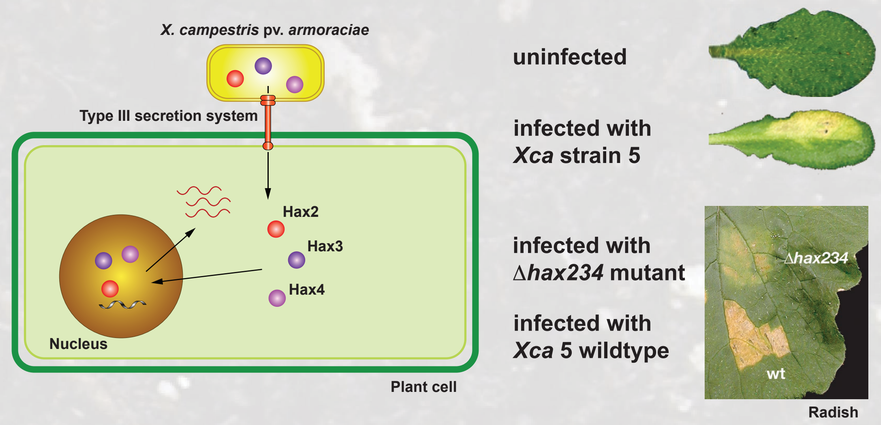
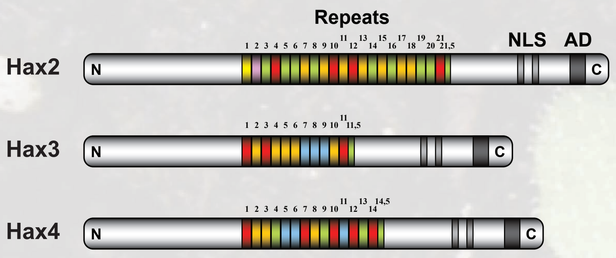
X. campestris pv. *armoraciae* - an Arabidopsis-pathogen

Xanthomonas campestris pv. *armoraciae* (*Xca*) is a *Brassicaceae* pathogen which infects the model plant *Arabidopsis thaliana*. *Xca* strain 5 translocates three effectors (Hax2, Hax3, Hax4) of the large AvrBs3/TAL (transcription activator-like)-effector family via a type III secretion system into plant cells. Together, the three *hax* genes are required for full pathogenicity of *Xca* strain 5 on radish plants (Kay *et al.* 2005). TAL effectors are important virulence factors which mimick eukaryotic transcription factors and induce expression of target genes in the plant cell nucleus. The specificity of TAL effectors is encoded in a central domain of tandemly arranged near-identical repeats which mediate direct binding to target promoters. **Here we solve how specificity of TAL effectors is encoded, predict target sequences for known TAL effectors and generate artificial TAL effectors with novel specificities.**



1 TAL effectors from *Xca*



Hax2: 35 aa repeats

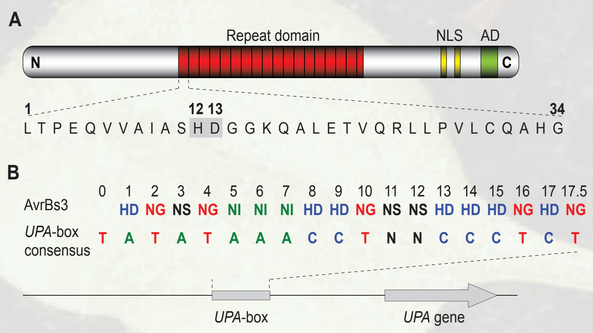
```
1                 12 13                35
LTPEQVVAIASHDGGKQALETVQRLLPVLCQAPHD
  RQ              NN                   A                   C
                  IG
                  NI
                  NG
```

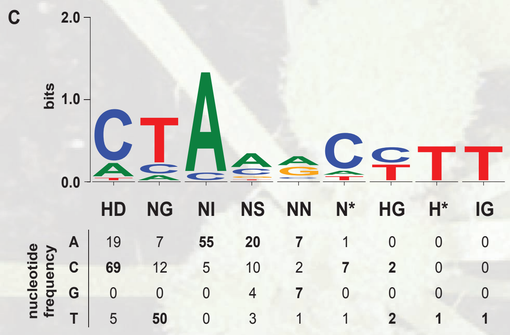
Hax3 & Hax4: 34 aa repeats

```
1                 12 13                34
LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHD
  Q               NG                   A
                  NS
                  NI
```

TAL effectors contain nuclear localization sequences (NLS) and an activation domain (AD) to function as transcriptional activators. DNA-binding is mediated by the central repeat domain, which is composed of near identical repeats of 34 or 35 amino acids length. We found that the hypervariable amino acids at position 12 & 13 mediate the specificity. **This revealed a general "code" for the DNA specificity of TAL effectors.**

2 Model for the DNA target specifity of TAL effectors

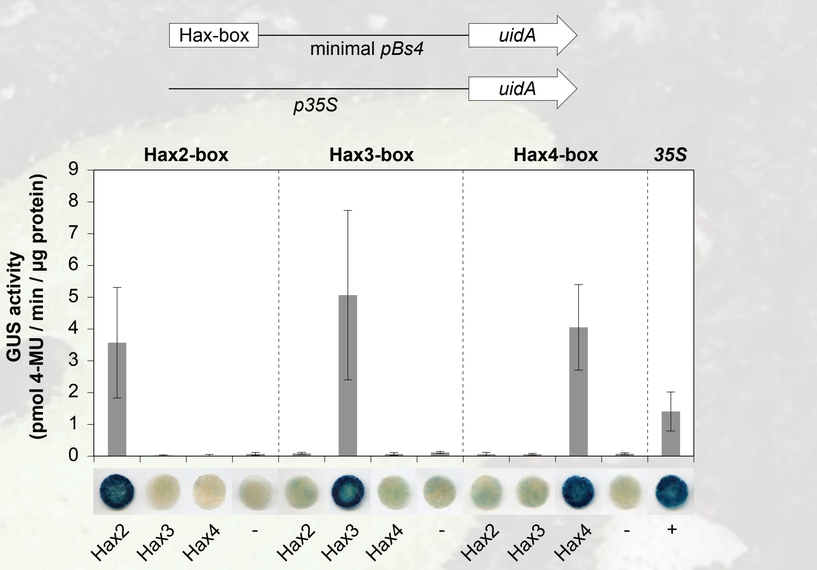




(A) TAL effectors contain central tandem repeats, NLSs, and an AD. Shown is the amino acid sequence of the first repeat of AvrBs3. Hypervariable amino acids 12 and 13 are shaded in gray. (B) Hypervariable amino acids at position 12 and 13 of the 17.5 AvrBs3 repeats are aligned to the UPA box consensus. **There is a correlation between the 12. &13. aa and the target box.**

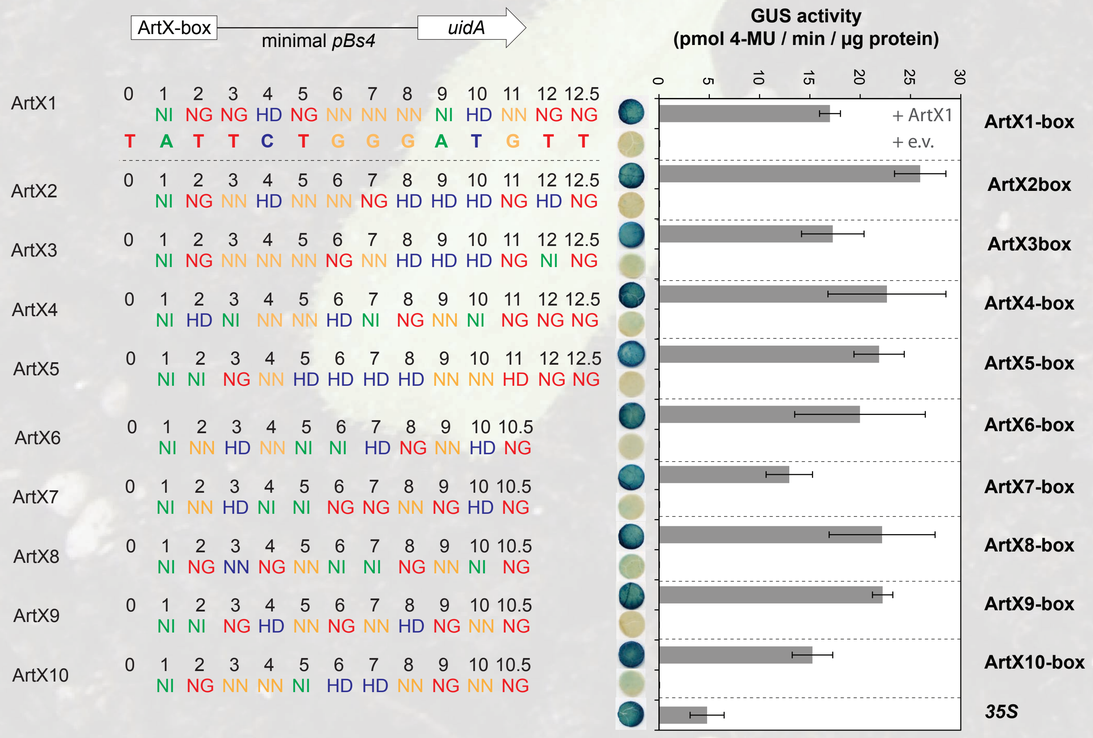
(C) Repeats of TAL effectors and predicted target sequences in promoters of induced genes were aligned manually. **Two hypervariable amino acids residues in each repeat recognize one base pair in the target DNA.**

3 Specific DNA targets for the TAL effectors Hax2, Hax3 and Hax4



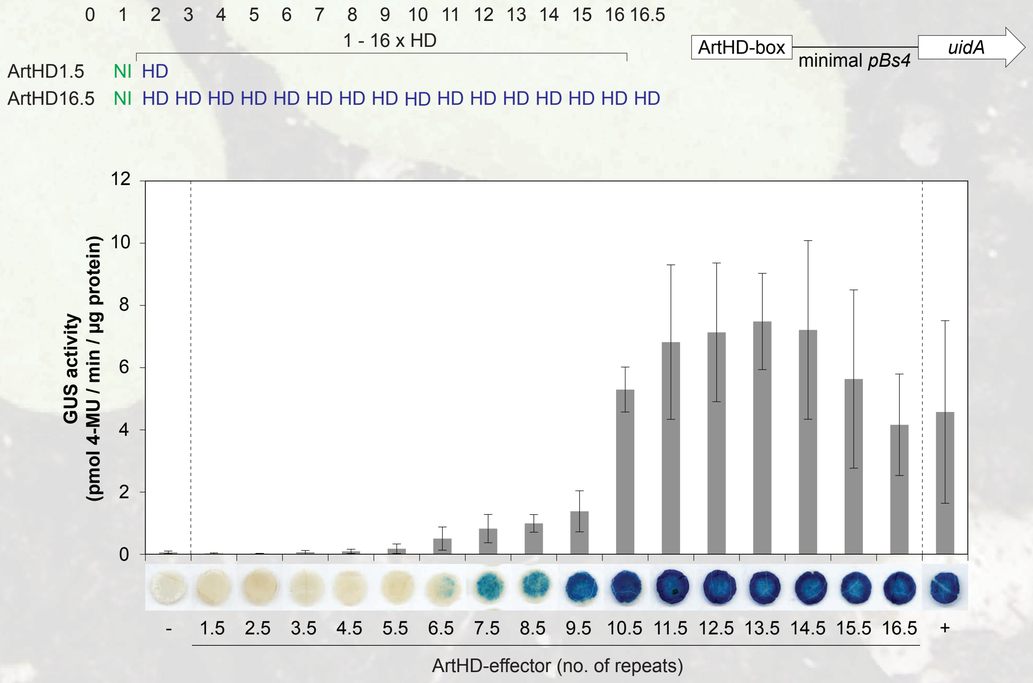
Target DNA-sequences for Hax2, Hax3, and Hax4 were predicted according to the code for DNA specificities of repeat types. Target boxes were combined with the minimal *Bs4* promoter which has weak basal activity, and inserted in a GUS (*uidA*)-reporter vector. Constructs were codelivered with *35S*-driven effector genes via *A. tumefaciens* into *N. benthamiana* and GUS activity determined. **This validates our model for DNA recognition by TAL effectors and the code for DNA target specificity of repeat types.**

4 Artificial TALs with novel specificities



Artificial effectors (ArtX) were generated with 12.5 and 10.5 randomly assembled HD-, NI-, NG-, and NN-repeats. Target boxes were predicted according to the code and inserted into the GUS reporter vector. Reporter constructs and 35S-driven *artX* genes were codelivered via *A. tumefaciens* into *N. benthamiana* and GUS activity determined. **This shows that we are able to design TAL effectors with DNA-binding domains that target a specific DNA sequence.**

5 Artificial TALs need a minimal number of repeats



Artificial TAL effectors were generated containing one NI-repeat and a varying number (1-16) of HD-repeats. Activation of a GUS-reporter containing a corresponding target box was analyzed after codelivery of effector genes and reporter via *A. tumefaciens* into *N. benthamiana*. **The data demonstrate that a minimal number of repeats is required to recognize the target box and activate gene expression. This also suggests that TAL effectors with fewer repeat numbers are largely inactive.**

Reference

Kay, S., Boch, J., and Bonas, U. 2005. Characterization of AvrBs3-like effectors from a Brassicaceae pathogen reveals virulence and avirulence activities and a protein with a novel repeat architecture. Mol. Plant-Microbe Interact. **18**:838-848.

Boch, J., Scholze, H. Schornack, S., Landgraf, A. Hahn, S., Kay, S., Lahaye, T., Nickstadt, A. and Bonas, U. 2009. Breaking the Code of DNA Binding specifity of TAL-Type III Effectors. Science **326**:1509-1512.

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