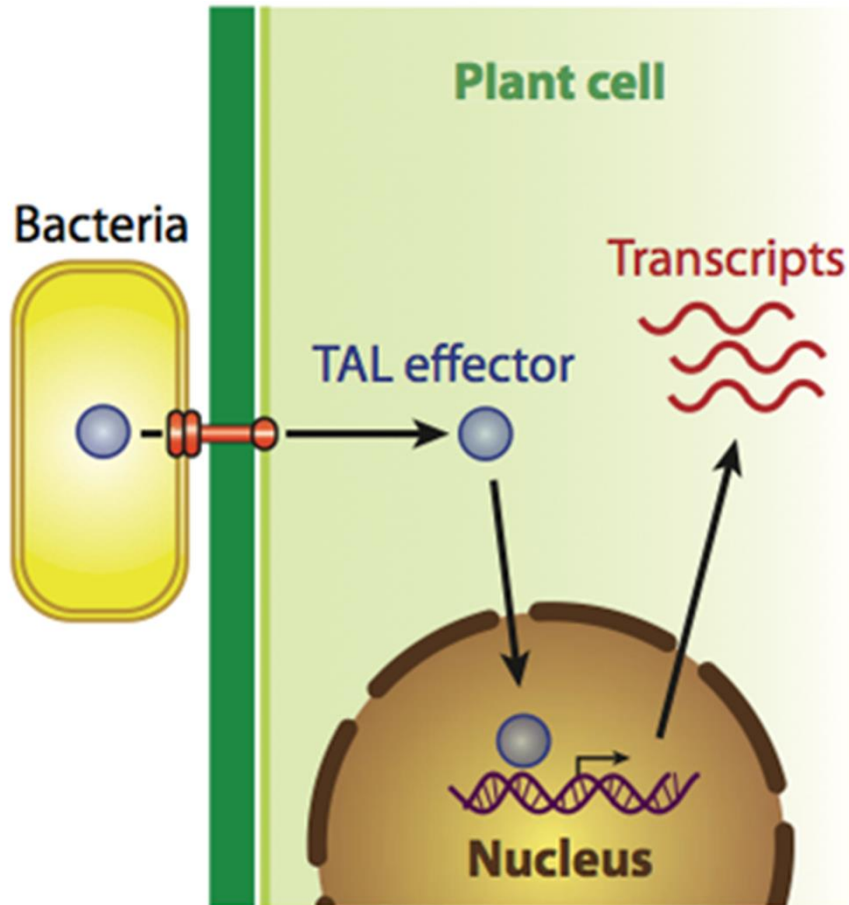


**TRANSCRIPTION ACTIVATOR-LIKE EFFECTORS (TALEs)
TALE-BASED TECHNOLOGIES
FOR TRANSCRIPTION ACTIVATION (TALE-TFs)**

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Basi Molecolari di malattie
03/10/2017

Transcription activator-like effectors (TALEs)

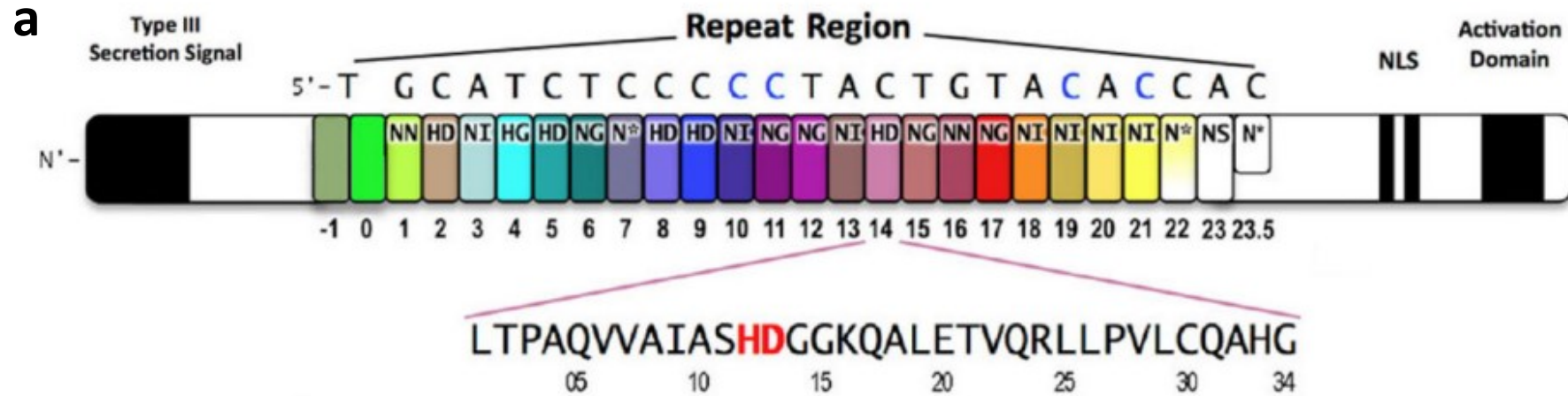


The first TAL effector identified was AvrBs3 from *Xanthomonas Campestris*, a pathogen of pepper.



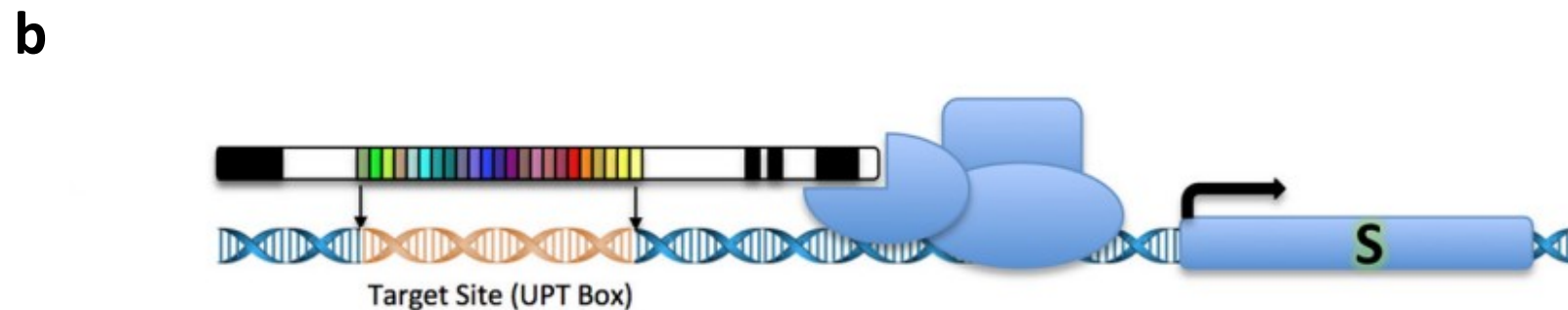
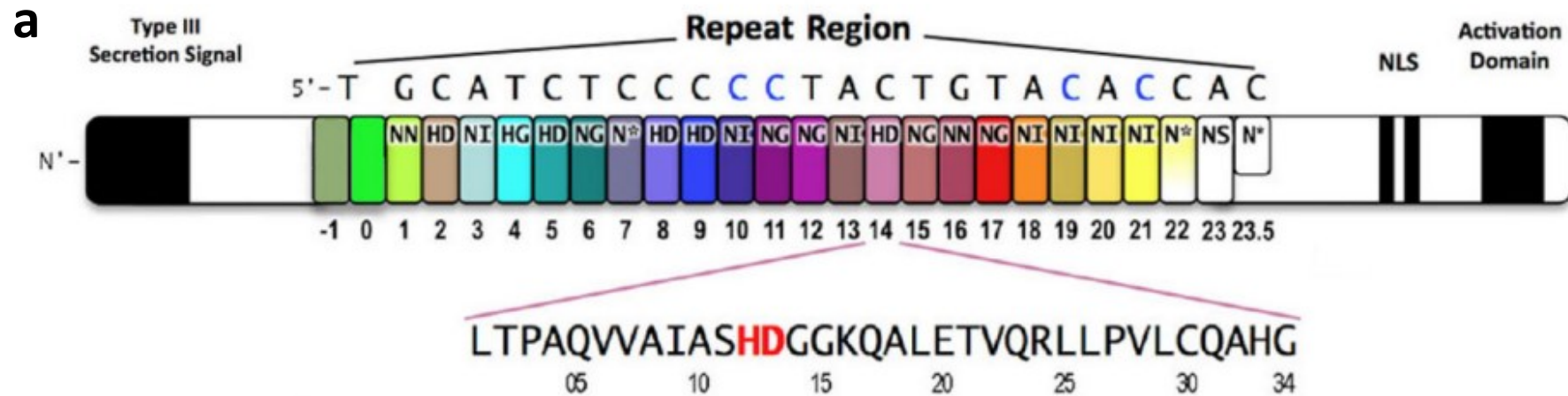
Xanthomonas AvrBs3 Family-Type III Effectors: Discovery and Function
Boch and Bonas, *Annu. Rev. Phytopathol.* 2010.

Tell me a tale of TALEs: protein structure



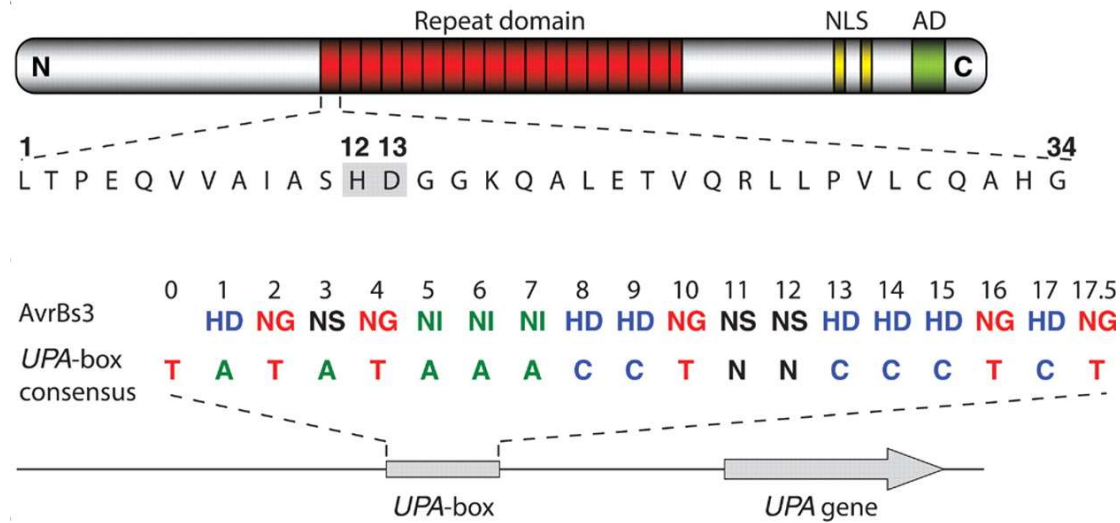
- a) TAL effectors contain N-terminal signals for bacterial type III secretion, variable numbers of tandem repeats that specify the target nucleotide sequence, nuclear localization signals, and a C-terminal region that is required for transcriptional activation.

Tell me a tale of TALEs: protein structure and function



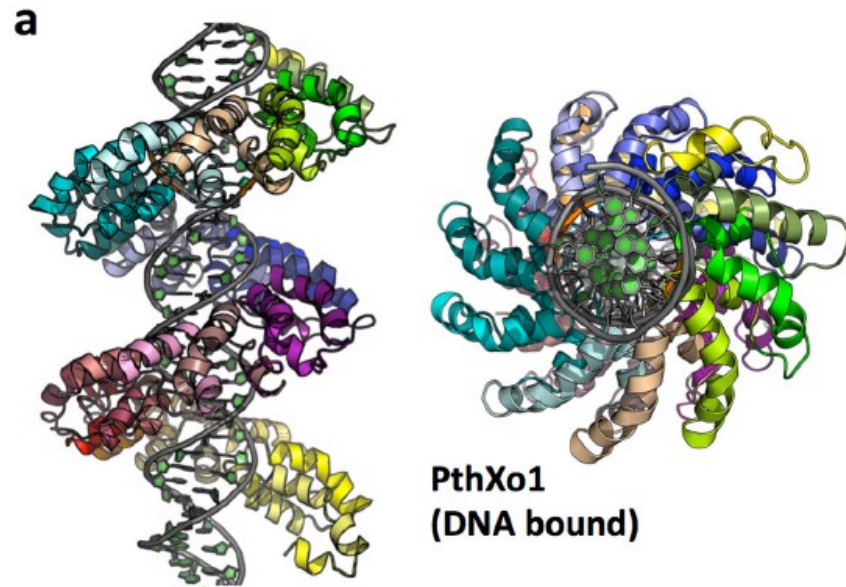
- TAL effectors contain N-terminal signals for bacterial type III secretion, variable numbers of tandem repeats that specify the target nucleotide sequence, nuclear localization signals, and a C-terminal region that is required for transcriptional activation.
- TAL effectors are translocated into the plant nucleus, where they bind to target sites located in the 5' promoter regions of genes that are subsequently activated.

Breaking the code of TALE-DNA binding specificity

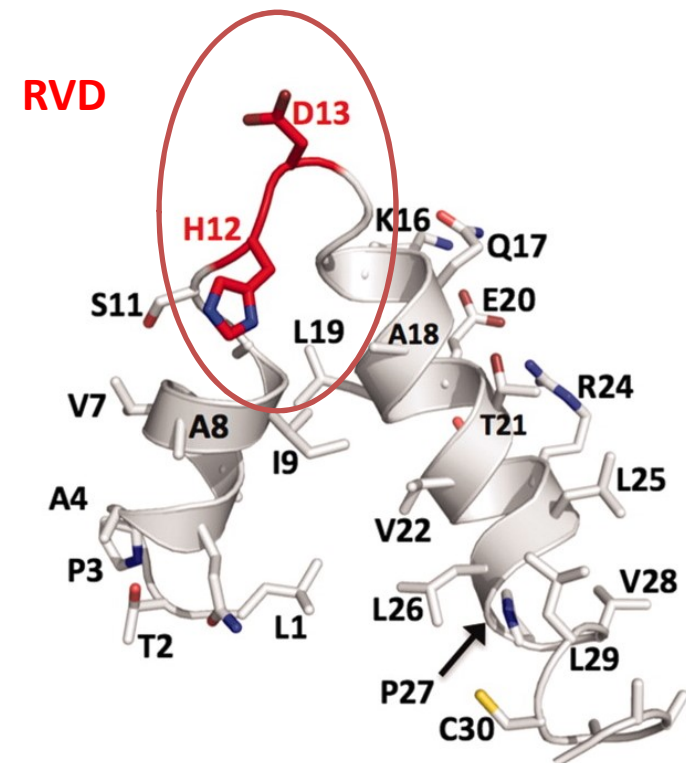
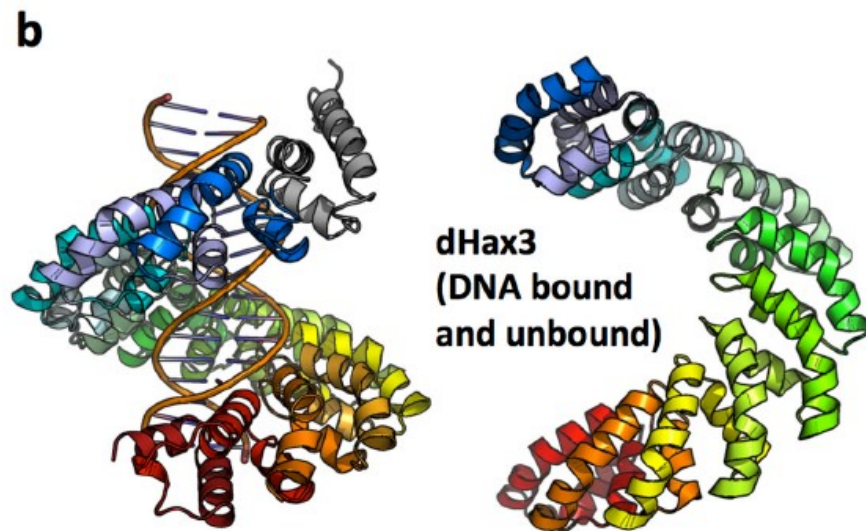


THE TALE CODE	
Di-Amino acid	Nucleotide bound
NI	= A
NG	= T
HD	= C
NN	= G/A

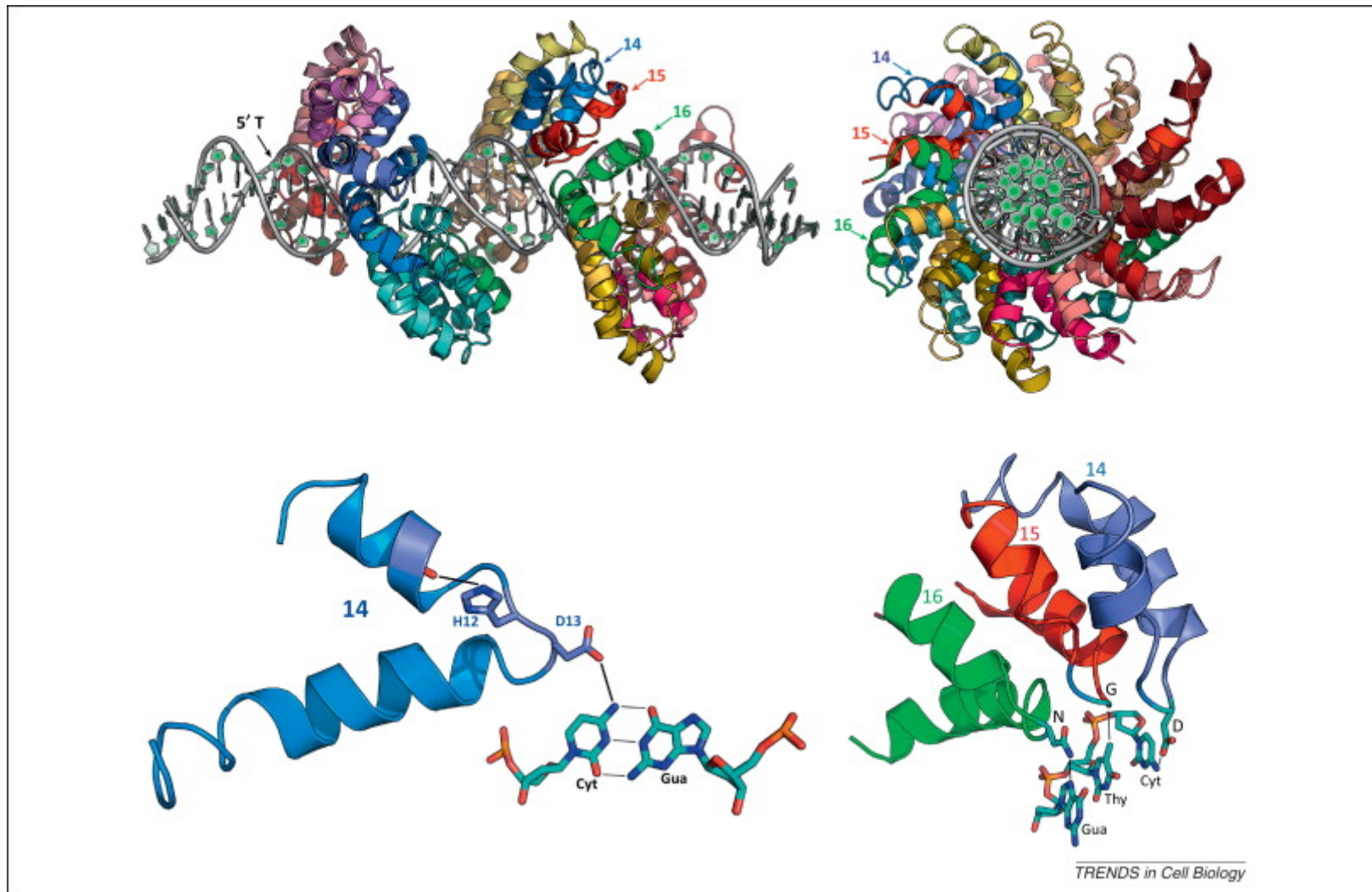
The structural basis for the DNA recognition “code”



Each TAL repeat forms a left-handed, two-helix bundle, in which the two hypervariable residues in each repeat (RVD) are found at the end of the loop that connects the two helices.



Recognition mechanism



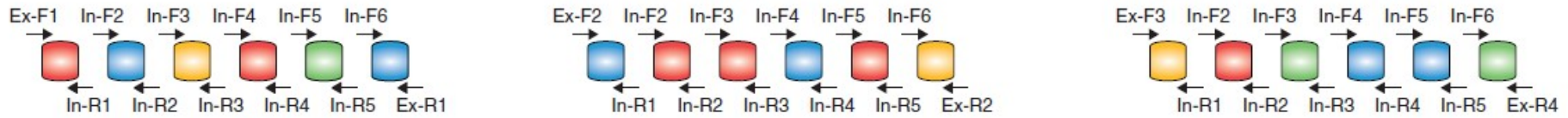
The sequence-specific contacts between the effector and the DNA are formed solely by the second residue of each RVD (at position 13 in each repeat) to atoms on the major groove edge of each base on a single contiguous strand of the DNA target.

APPLICATIONS

Genome modifications	Description	Genome editing tools
Gene tagging	Add a fusion tag (e.g. luciferase, GFP) to track an endogenous promoter activity or an endogenous protein expression and location	TALEN
Gene mutagenesis	Introduce point mutations to an endogenous gene	TALEN or CRISPR
Gene knockout	Introduce deletions or insertions (e.g. a selection marker) to knockout an endogenous gene	TALEN or CRISPR
Gene activation	Activate an endogenous gene expression	TALE-TF or CRISPR-TF
Gene repression	Repress an endogenous gene expression	TALE-R or CRISPR-R
Safe harbor knockin	Knockin an exogenous ORF or other genetic element to safe harbor sites of human or mouse genome	TALEN or CRISPR

Construction of TALE DNA-binding domains

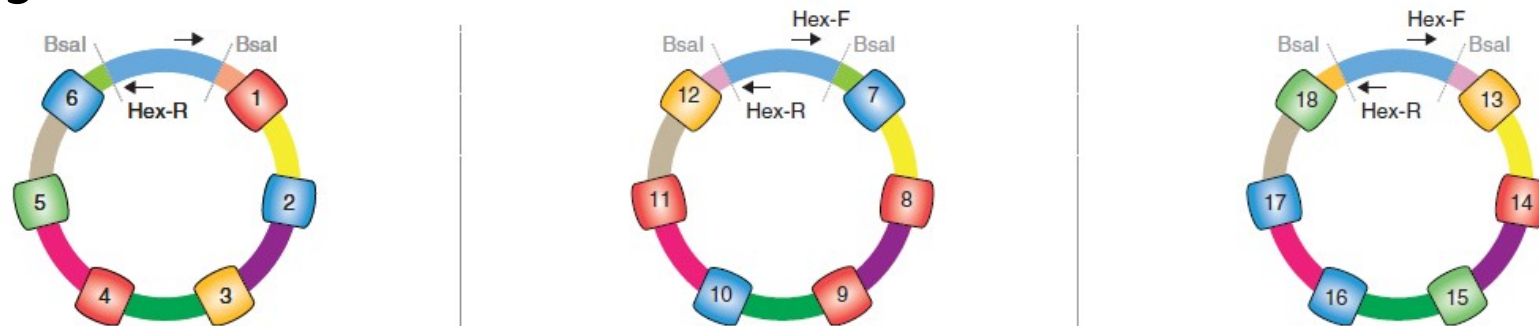
Stage 1



Stage 2

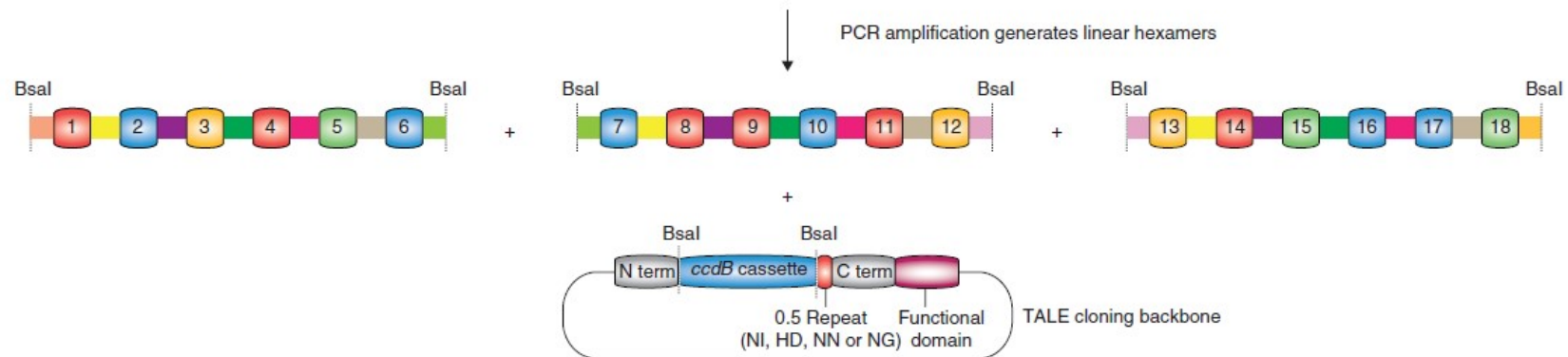


Stage 3

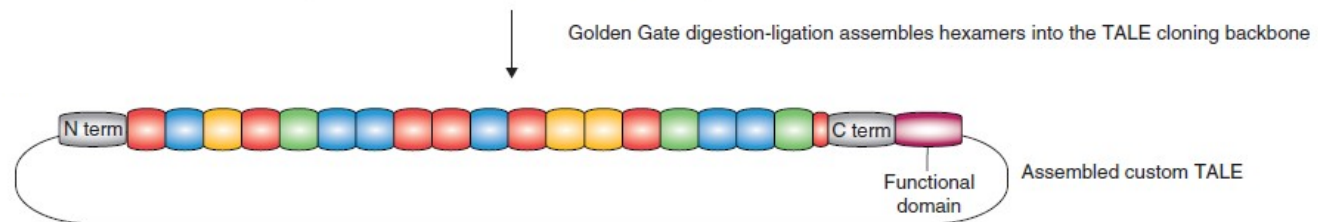


Construction of TALE DNA-binding domains

Stage 4



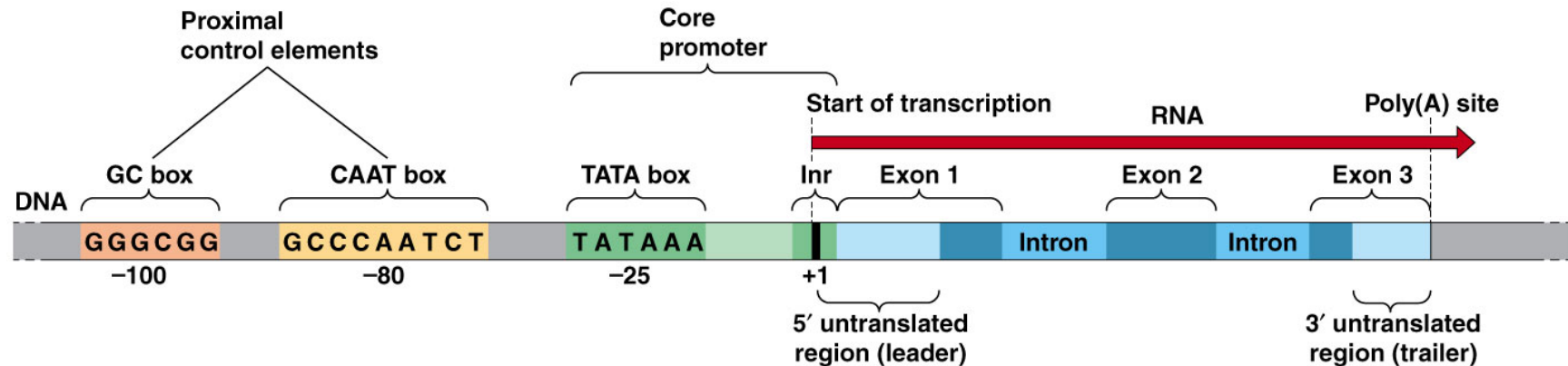
Stage 5



Deep inside the Golden Gate cloning:

<http://synbio.tsl.ac.uk/docs/item/62c207a9-e633-4e3c-b61f-5ff133be31ad>

Eukaryotic promoter overview



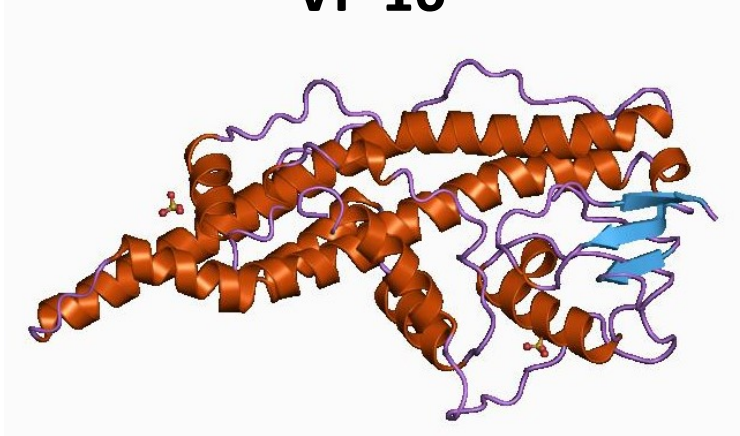
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The **promoter of a eukaryotic gene** can be defined as a **sequence** that sets the transcription start site for RNA polymerase. Strong promoters contain an **A/T rich** sequence known as the **TATA box located 26-31 bp upstream** of the start site.

Other genes have alternative sequence elements known as initiators (Inr) which also serve as promoters that set the RNA Pol II start site. Finally, CG-rich repeat sequences (CpG islands) are used by RNA Pol II as promoters in 60-70% of genes.

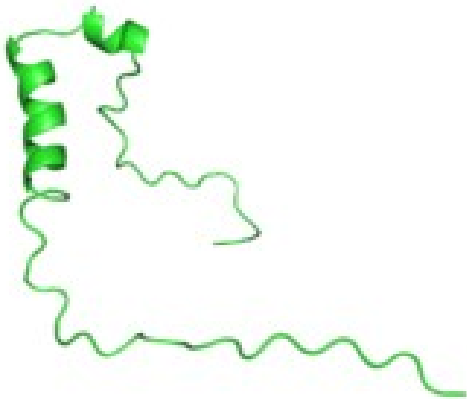
Transcription Activator/Repressor can be engeneered

VP 16



Virion protein 16 (VP16) of herpes simplex virus type 1 is a potent transcription activator able to form complex with host transcription factors and induce early transcription of the herpes simplex viruses.

KRAB



Krüppel associated box (KRAB) is a human transcriptional repressor domain present in more than 400 human zinc finger transcription factors.

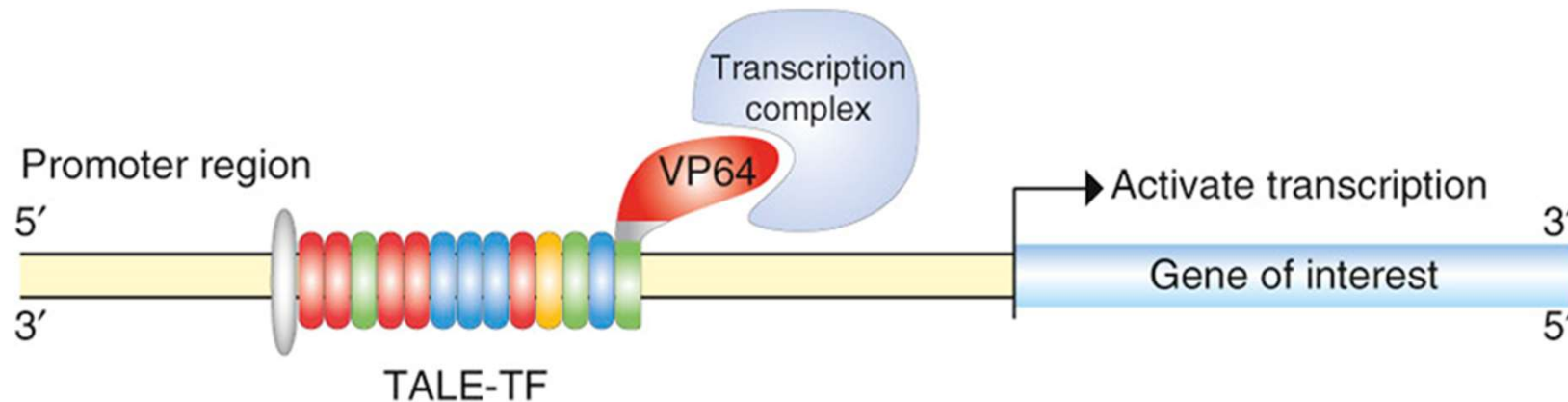
It is the strongest repressor in the human genome.

Both are used fused to elements able to target specific regions of DNA (as TALE proteins!)

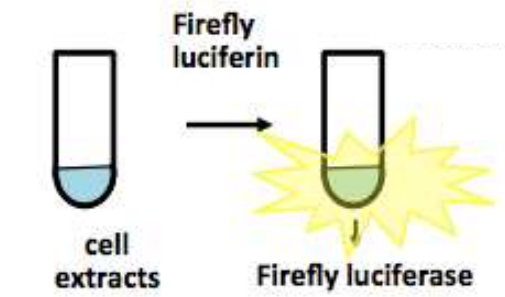
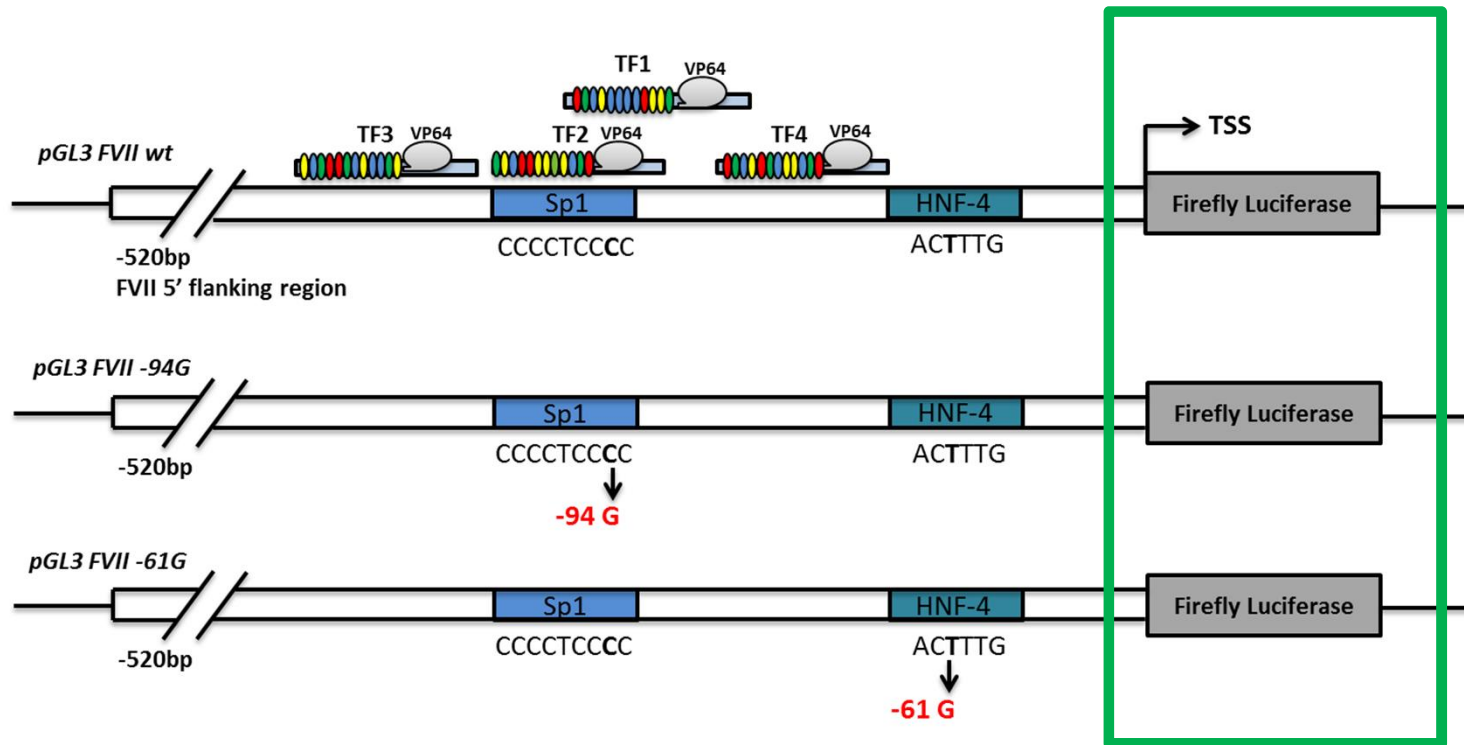
Engineered TALE-Transcription Factors (eTFs)



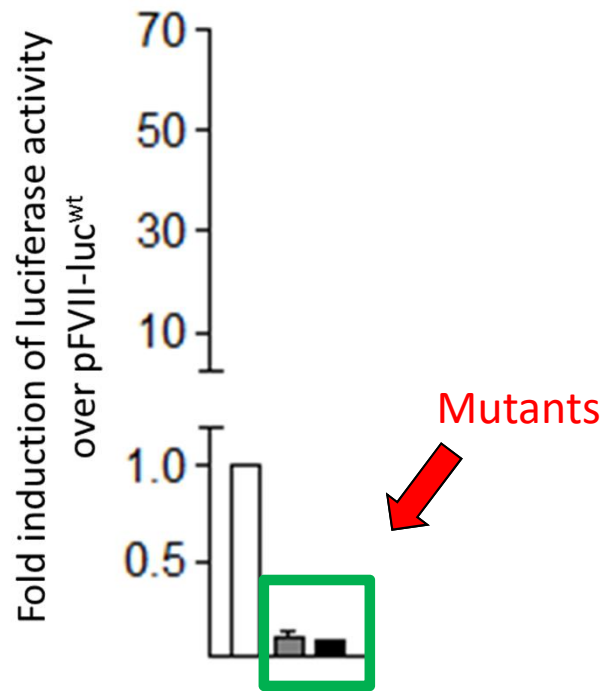
The TALE DNA binding domain is fused with a nuclear localization signal (NLS) and with the VP64 synthetic activator domain to induce transcription.



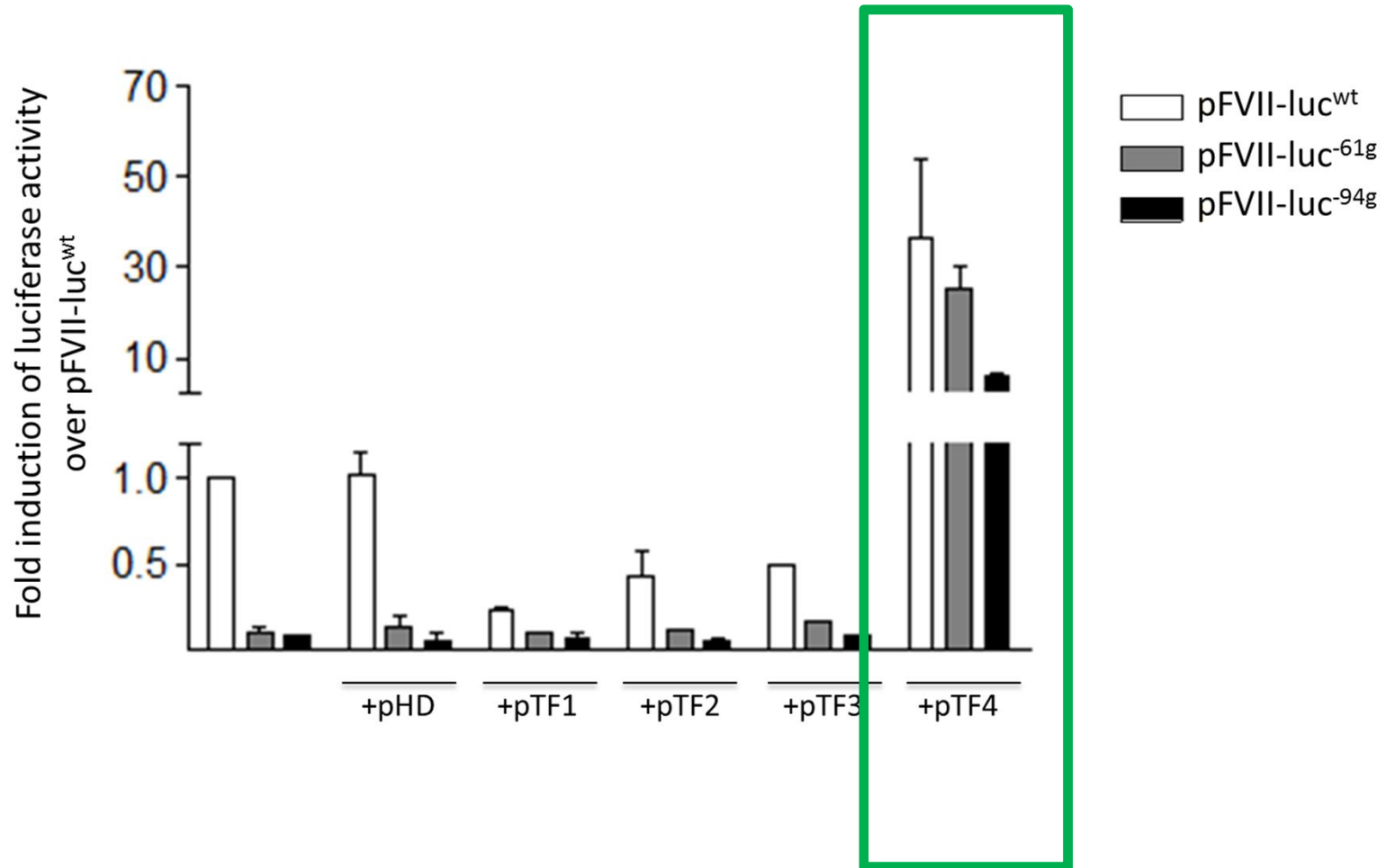
Experimental approach: reporter vectors and TALE TF design



Is the TALE TF system able to rescue the impaired transcription of the mutants?

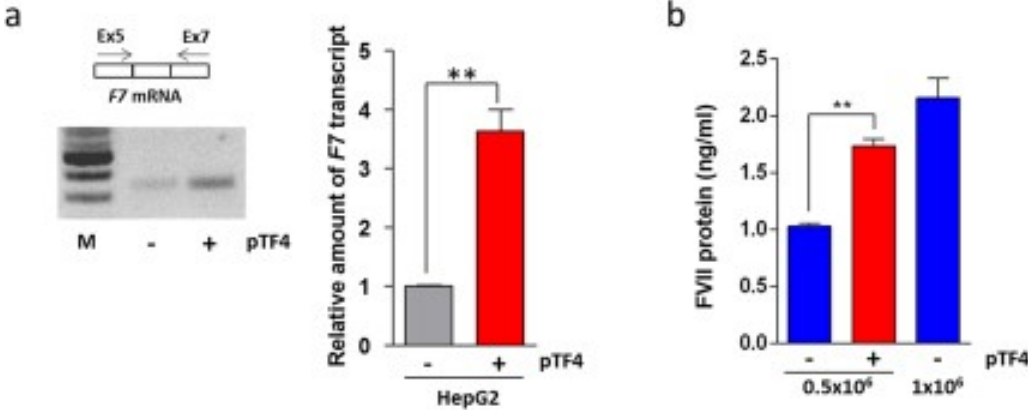


Is the TALE TF system able to rescue the impaired transcription of the mutants? **YES**



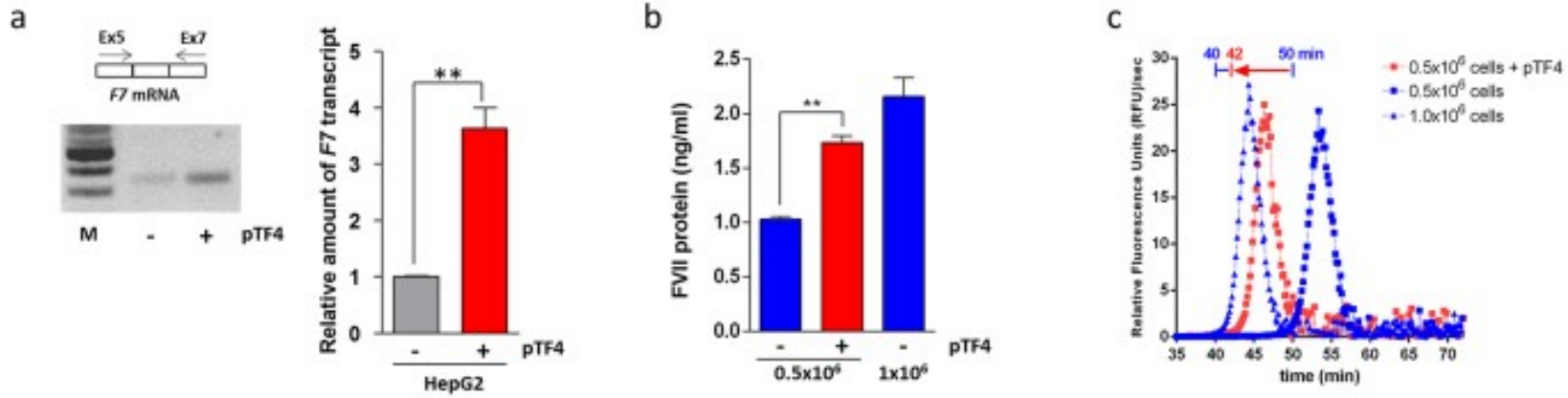
TF4 trans-activation activity on the F7 gene

HepG2



TF4 trans-activation activity on the F7 gene

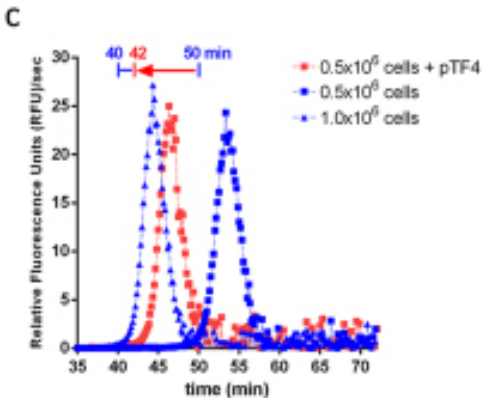
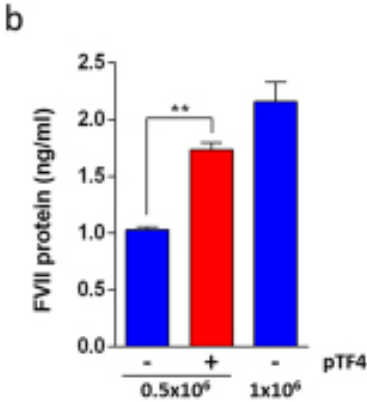
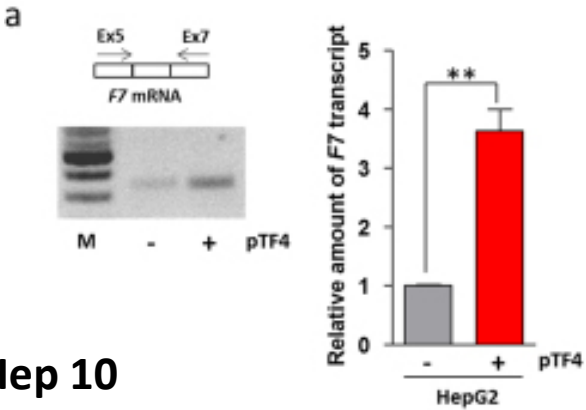
HepG2



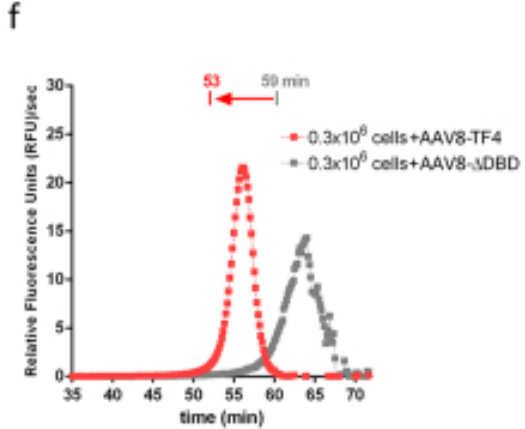
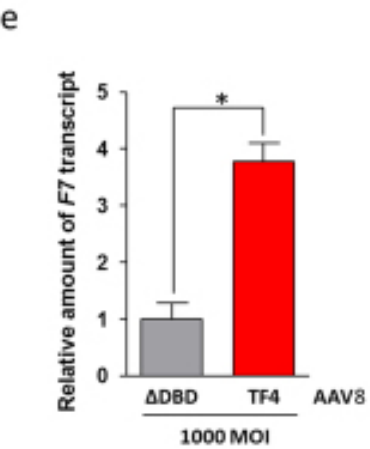
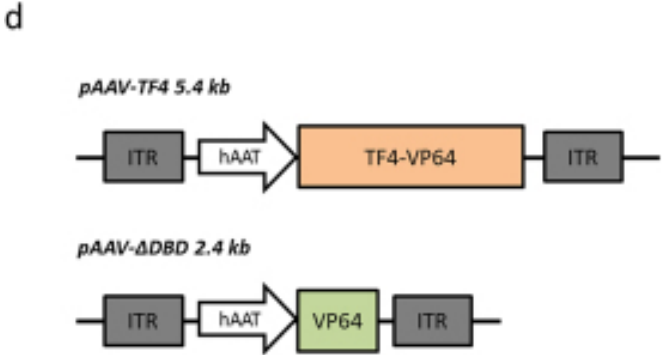
Lag time: **time** to start

TF4 trans-activation activity on the F7 gene

HepG2



Hep 10



Conclusions:

- ❑ TALE proteins can be engineered to bind specific regions in the genomic DNA
- ❑ In molecular biology these proteins can be used both for genome engineering and transcription activation/repression.
- ❑ We created a TALE-TF able to specifically rescue and enhance or trigger the FVII promoter activity.
- ❑ This is the proof of the efficacy of TALE-TF to rescue gene expression also in presence of two different disease-causing promoter mutations.
- ❑ Moreover, if delivered in vivo, these results could open new therapeutic strategies for coagulation factors as well as other genetic diseases sharing similar pathogenic mechanisms.

