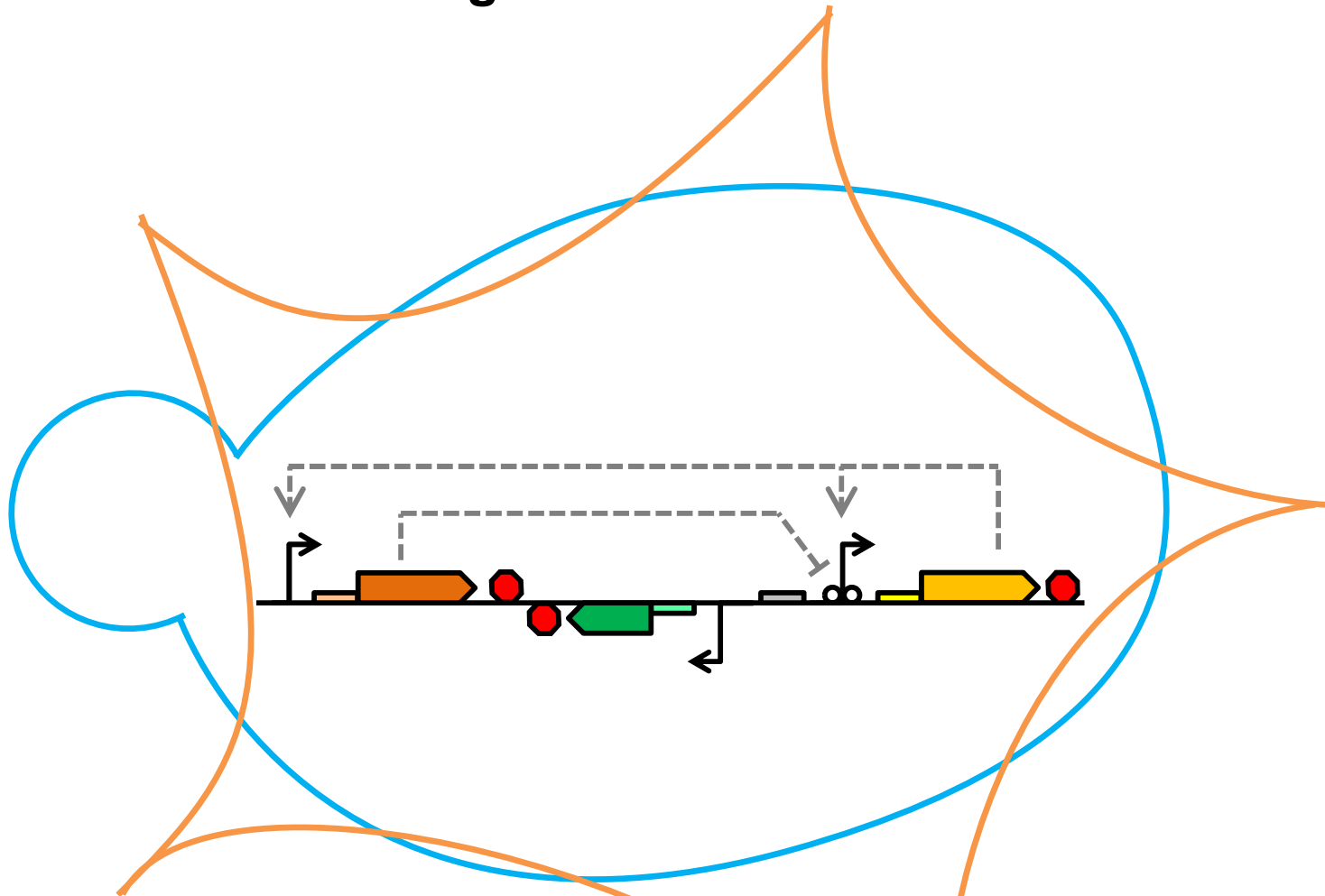


Synthetic Circuits in Eukaryotes

UE2.1 Biological Parts and Devices

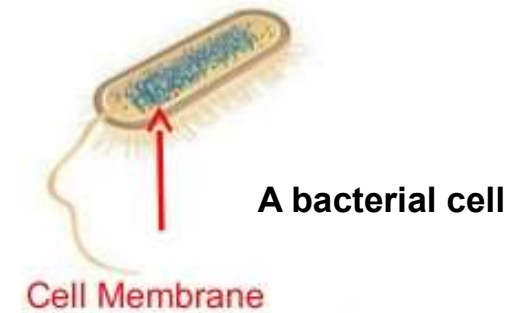
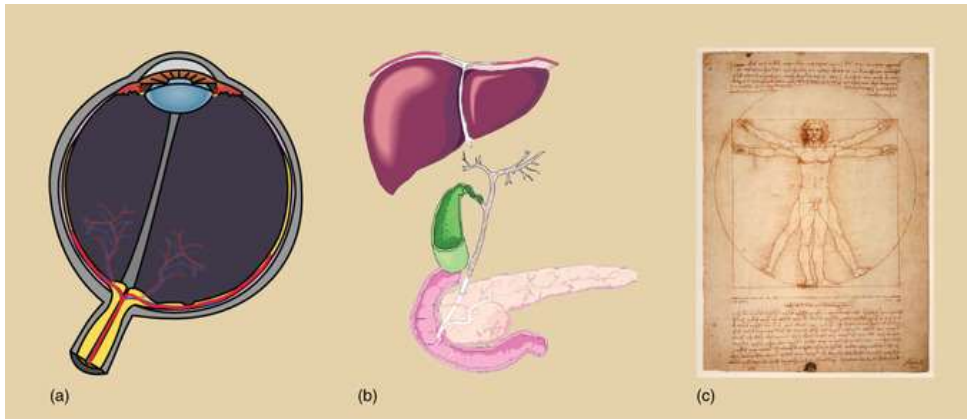


Manish Kushwaha

10 October, 2024

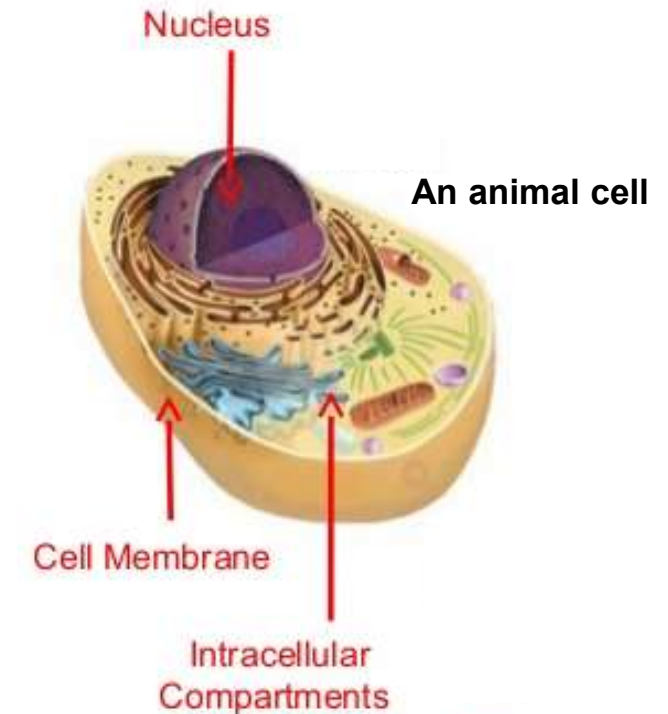
Eukaryotic cells are bigger and more complex

- Eukaryotes have many cells and cell-types, as well as many cellular compartments



A bacterial cell

Cell Membrane



An animal cell

Cell Membrane

Intracellular
Compartments

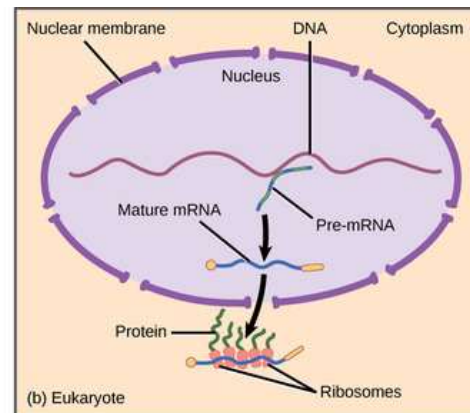
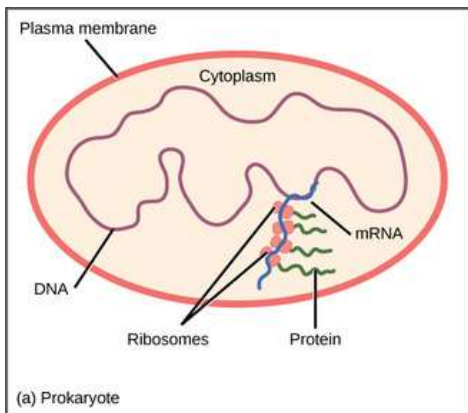


Image source/s:

<https://courses.lumenlearning.com/boundless-biology/chapter/regulation-of-gene-expression/>

<https://pt.slideshare.net/legoscience/cell-basics>

Eukaryotic cells are bigger and more complex

- Eukaryotic **transcription and translation are NOT coupled**
- **Transcription:**
 1. Promoter length and complexity
 2. Chromatin accessibility/ transcriptional bursts (3D nuclear positioning)
 3. mRNA maturation and export

- **Translation:**
 1. mRNA stability
 2. Ribosomal scanning

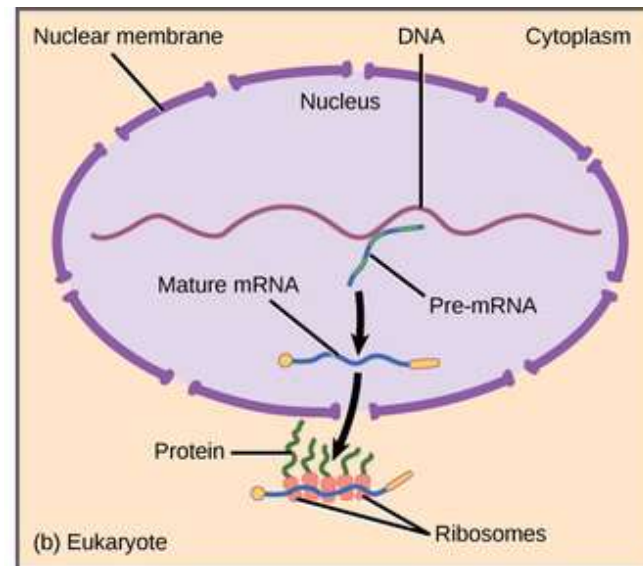
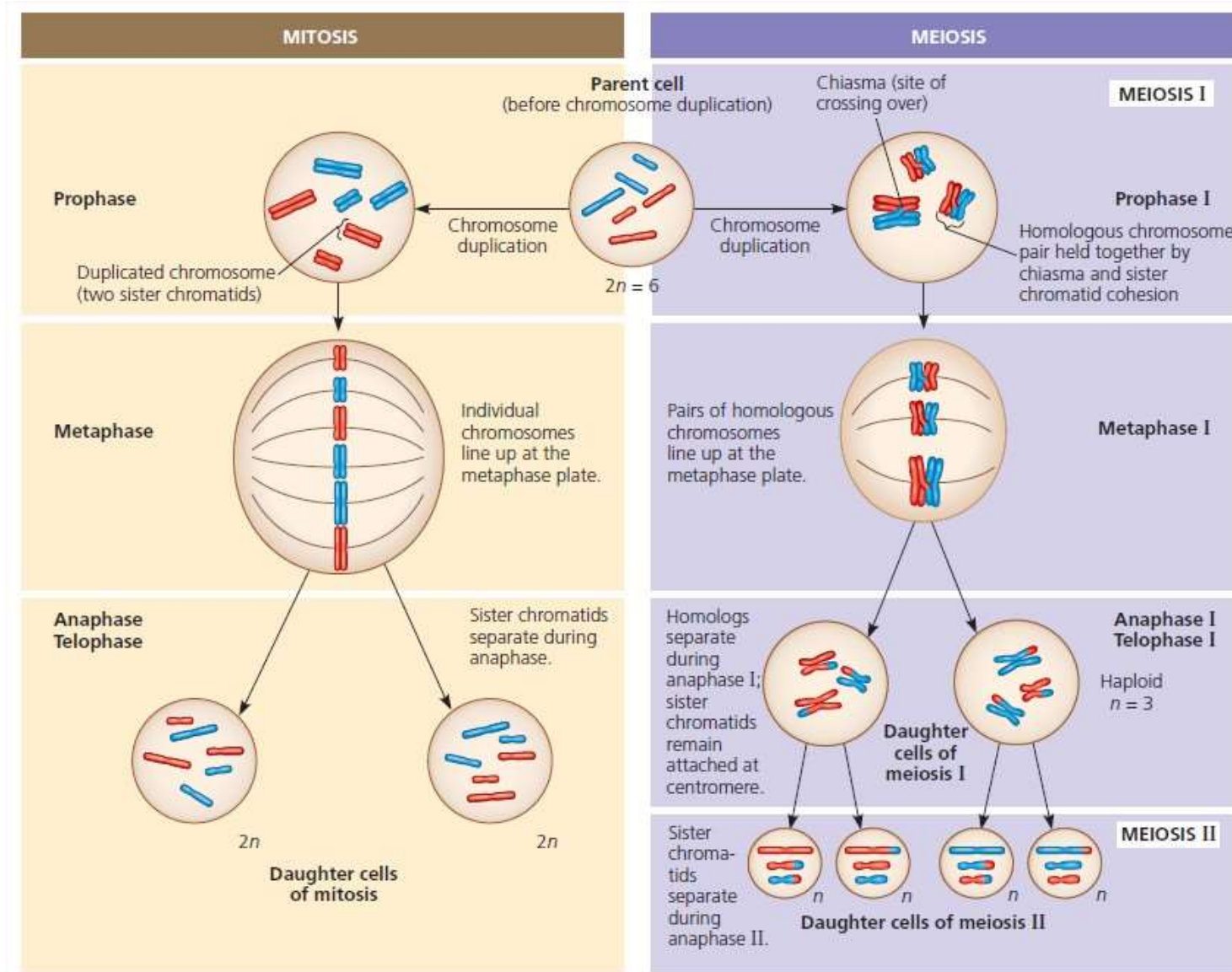


Image source/s:

<https://courses.lumenlearning.com/boundless-biology/chapter/regulation-of-gene-expression/>

Eukaryotic cells are bigger and more complex

- Eukaryotes have a well-defined cell cycle



Open vs closed mitosis (Boettcher & Barral, 2013. Nucleus.)

Image credit: Yoan Cahyono

<https://yoanx7.blogspot.com/2013/05/a-comparison-of-mitosis-and-meiosis.html>

Eukaryotic transcription

- Eukaryotic transcription: **3 different polymerases**
- Higher complexity of promoter interactions
- Chromatin roadblocks

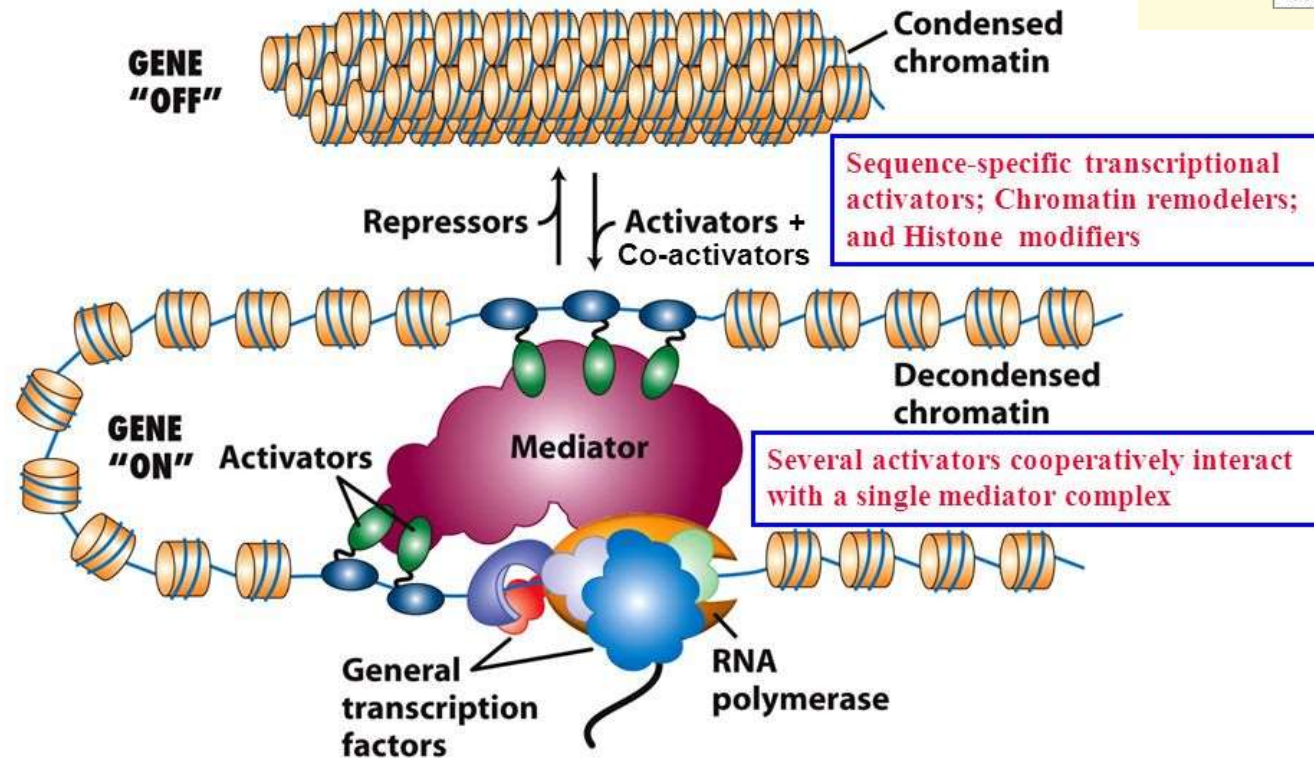
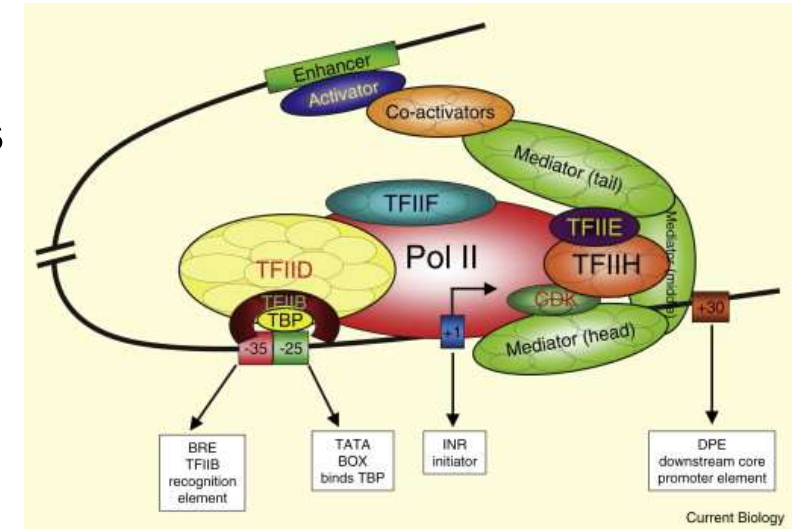
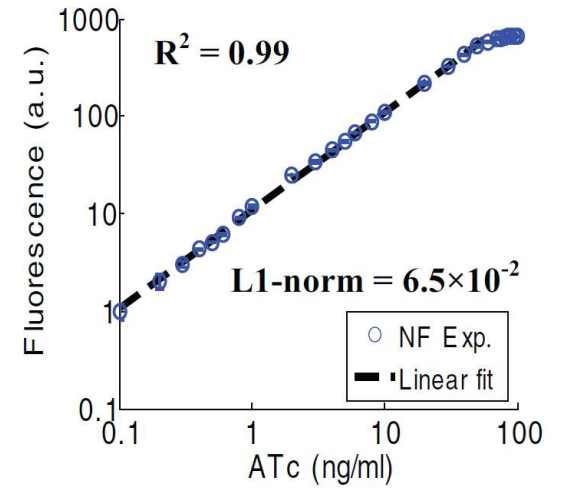
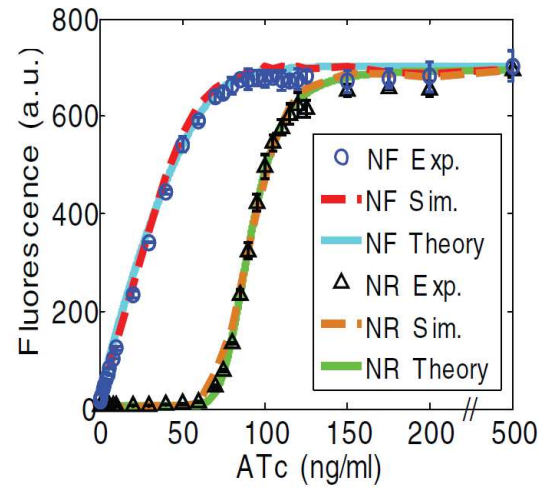
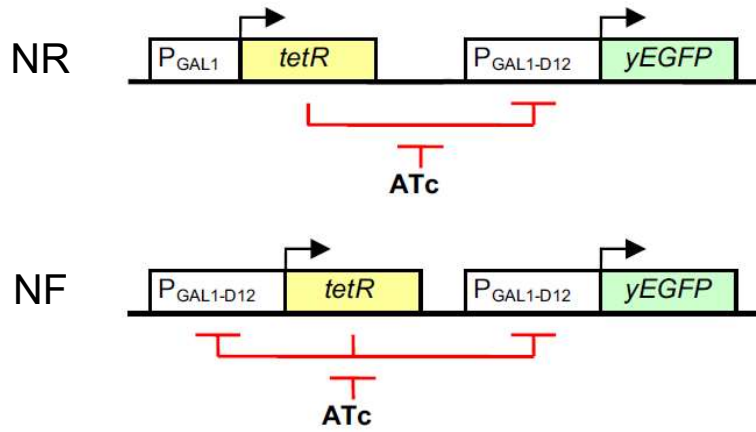


Image source/s:

[https://www.cell.com/current-biology/fulltext/S0960-9822\(08\)01569-8](https://www.cell.com/current-biology/fulltext/S0960-9822(08)01569-8)

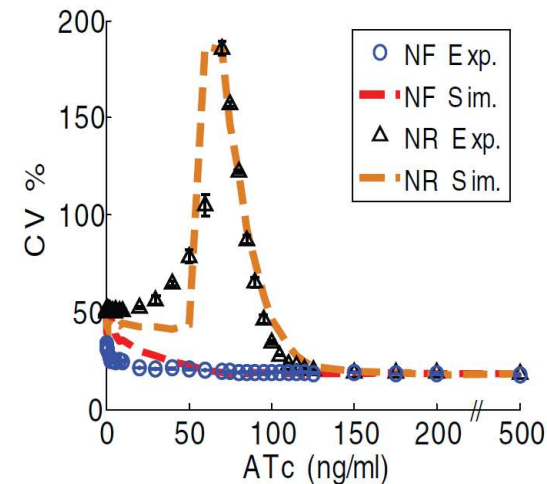
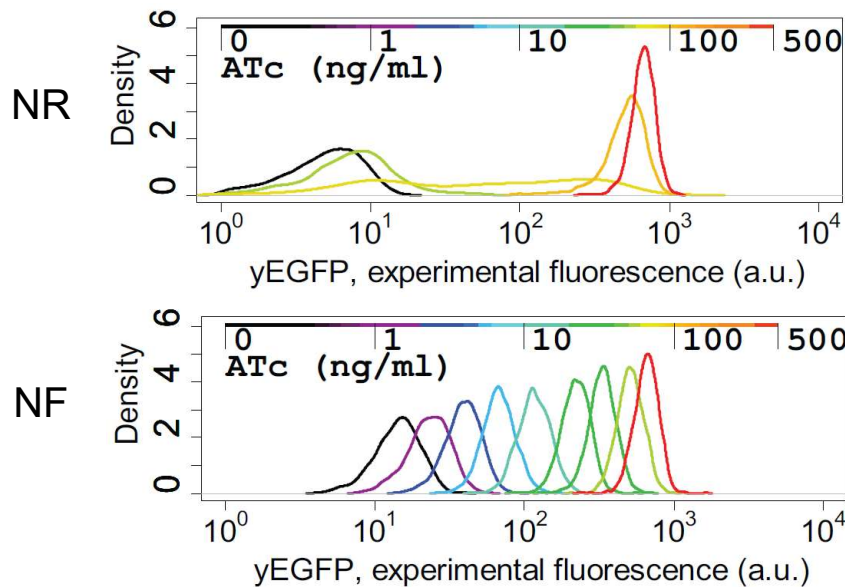
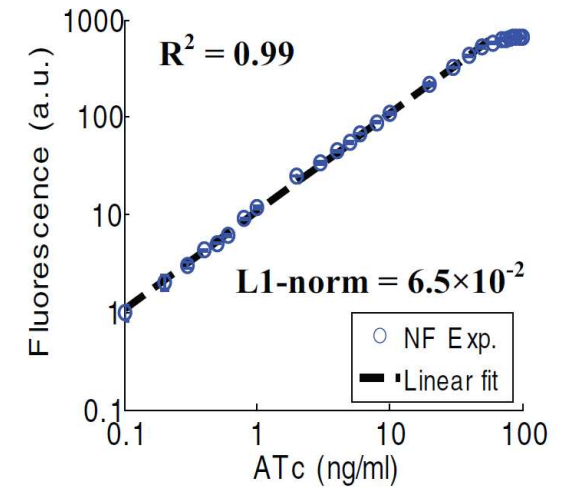
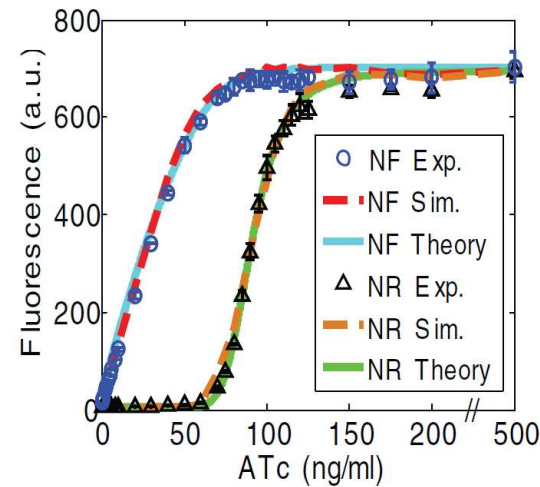
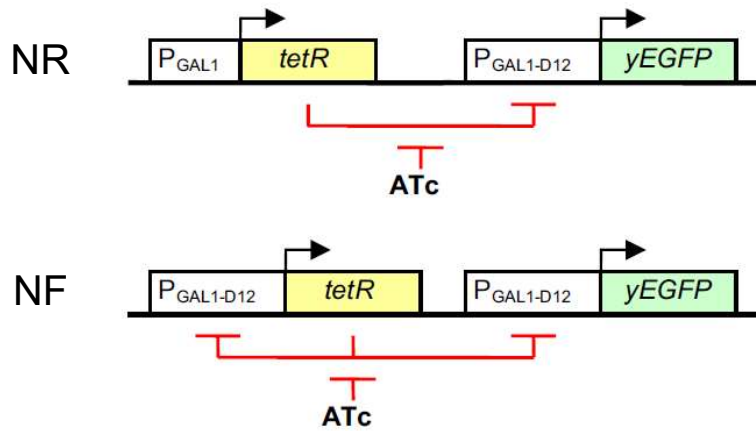
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Feedback control linearizes Response Curve in Yeast



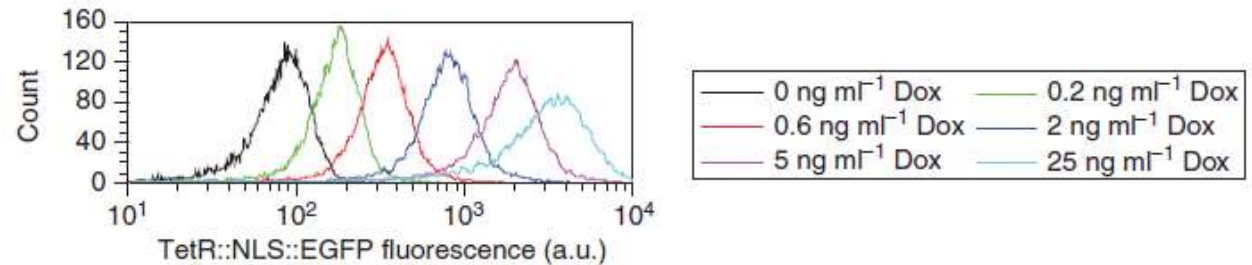
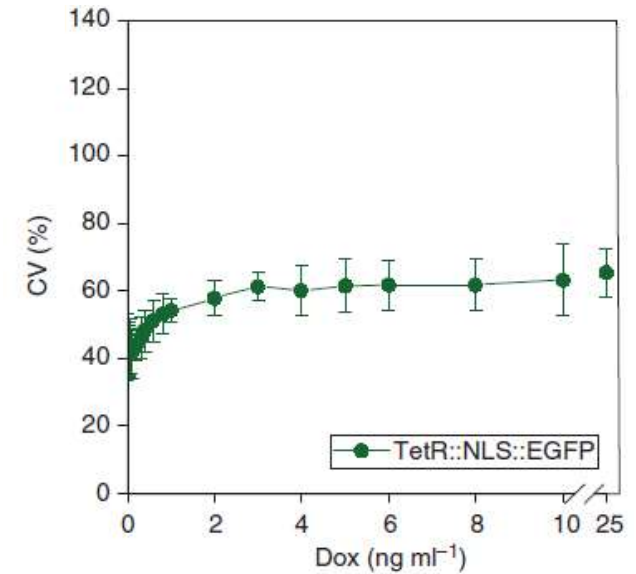
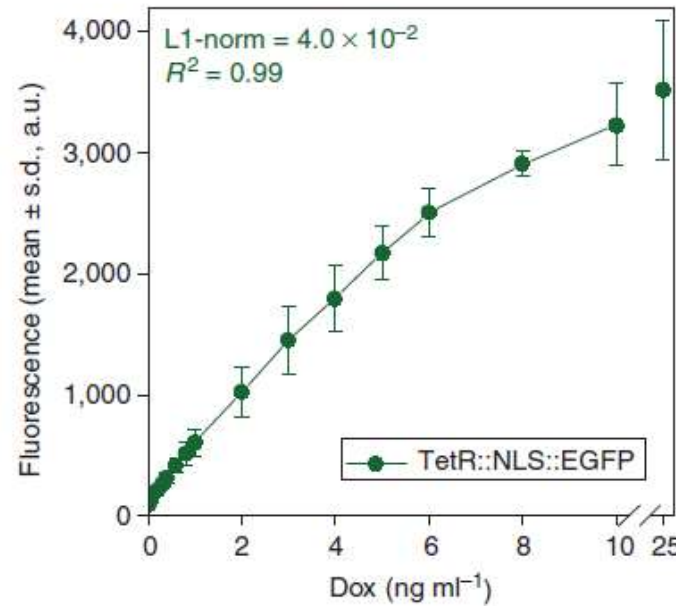
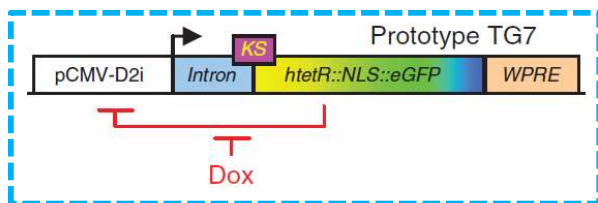
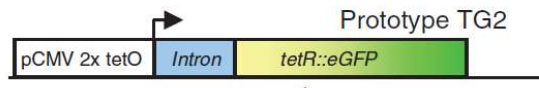
- Negative Feedback (NF) linearizes response curve

Feedback control linearizes Response Curve in Yeast



- Negative Feedback (NF) linearizes response curve, and reduces heterogeneity of expression

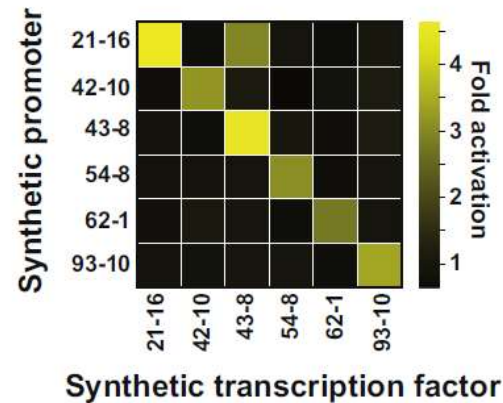
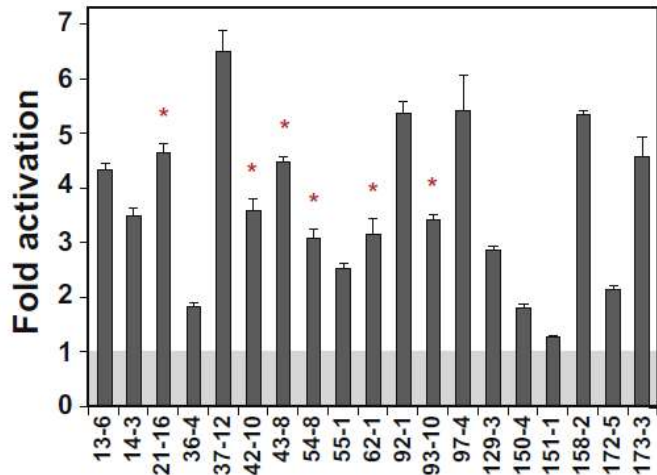
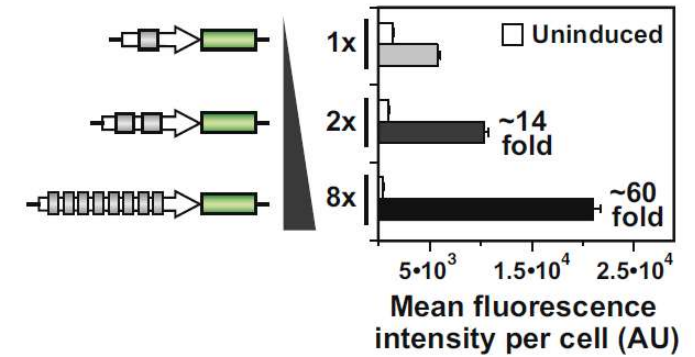
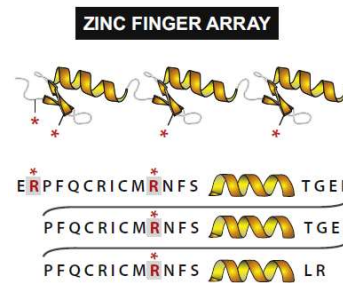
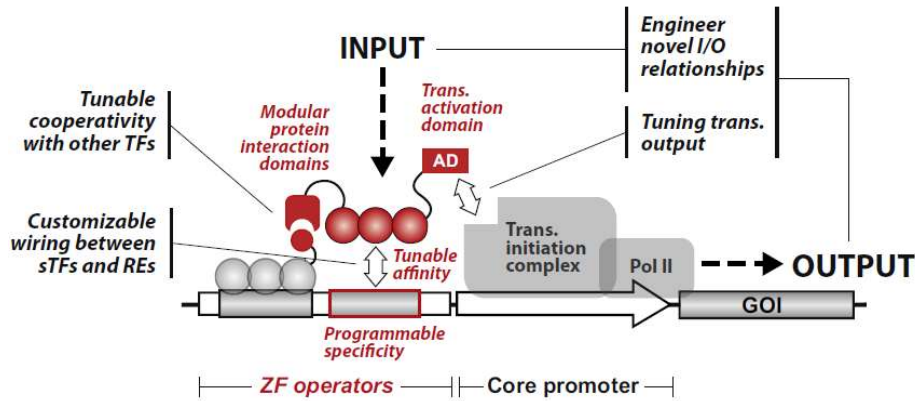
Porting a Yeast circuit to Mammalian cells



- Multiple optimizations were needed to adapt a yeast “linearizer” circuit to human cells (HEK293, MCF-7).

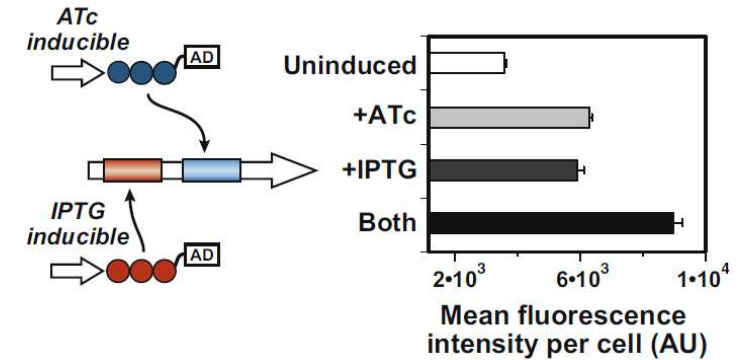
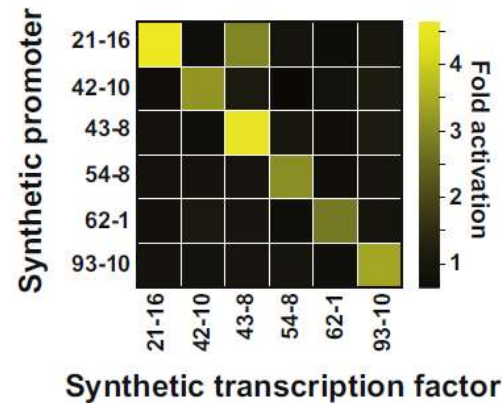
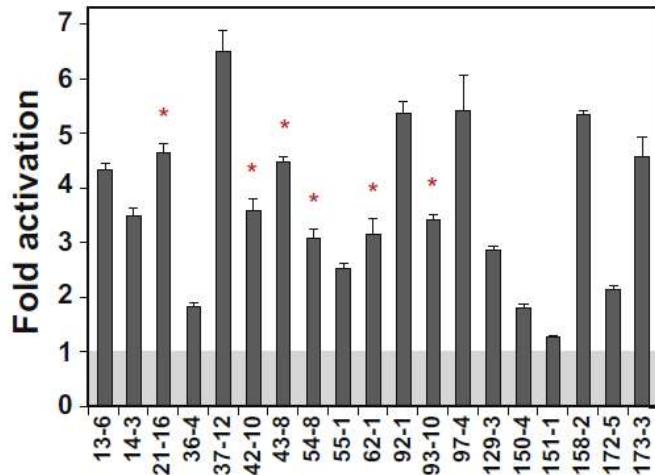
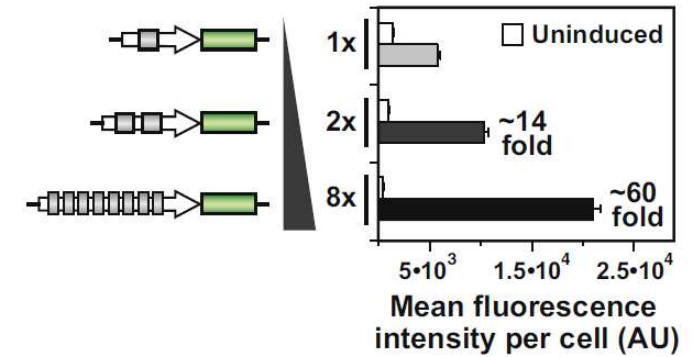
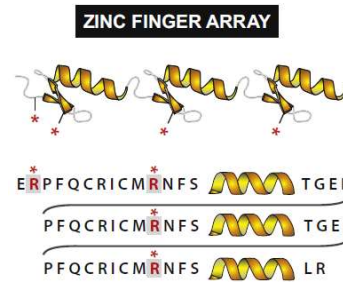
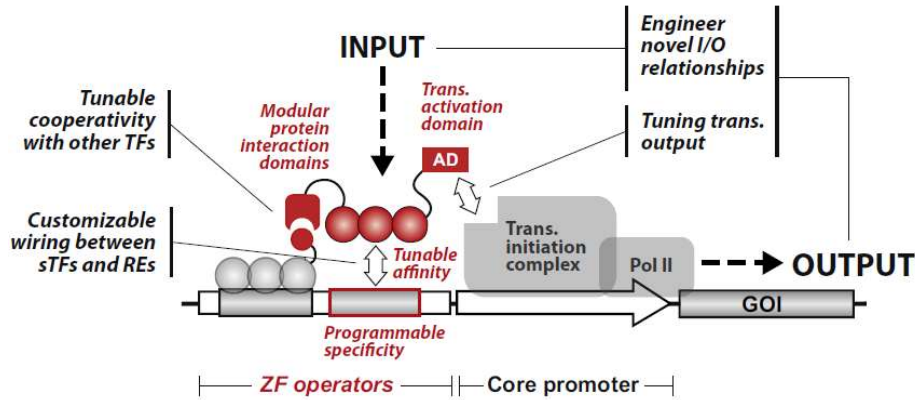
(Nevozhay *et al.*, 2013. Nat. Commun.)

Rational Design of Synthetic Yeast Promoters



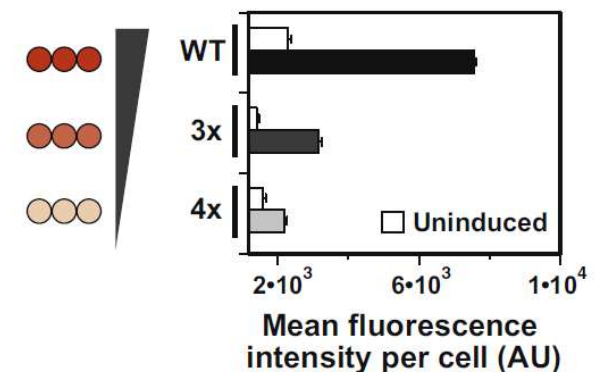
- Tuning Yeast promoters through synthetic transcription factors: fusion of **ZNF motif** and trans-activating **VP16** domain.
- Each ZNF domain is **30 aa** long and forms a $\beta\beta\alpha$ configuration, where α -helix AAs interact with **3 nts** in the major groove of DNA.

Rational Design of Synthetic Yeast Promoters

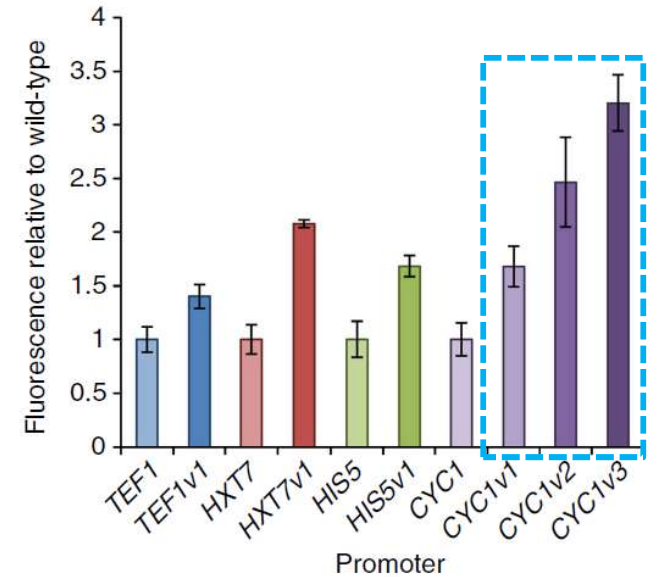
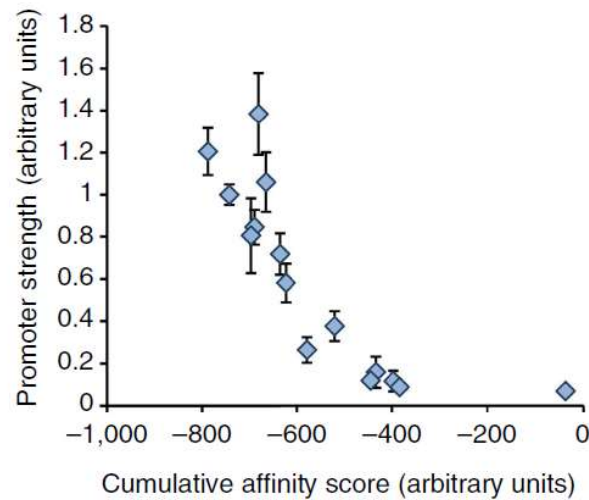
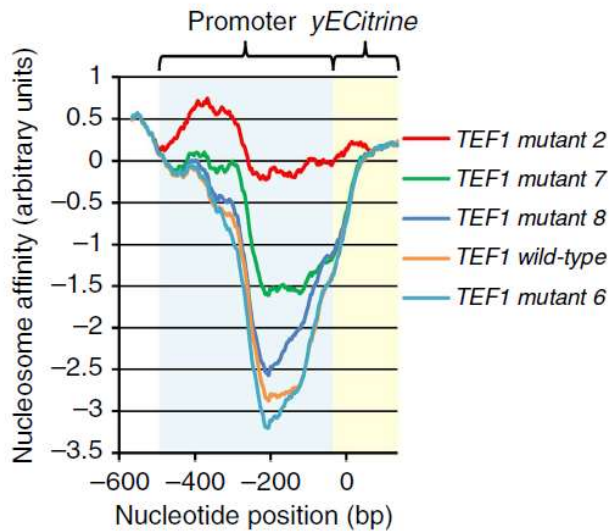
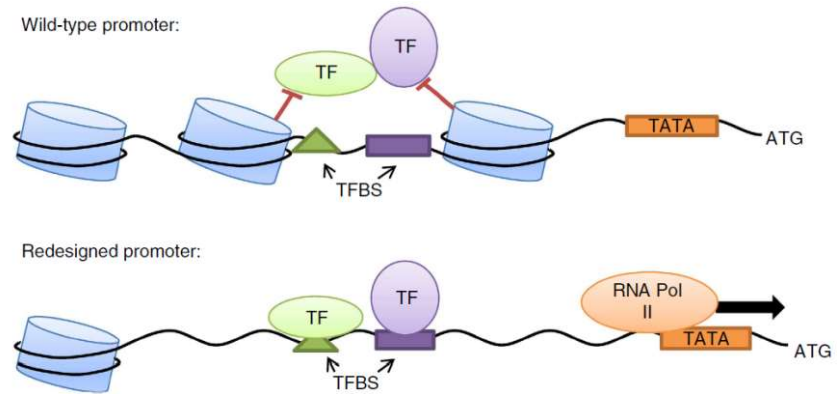
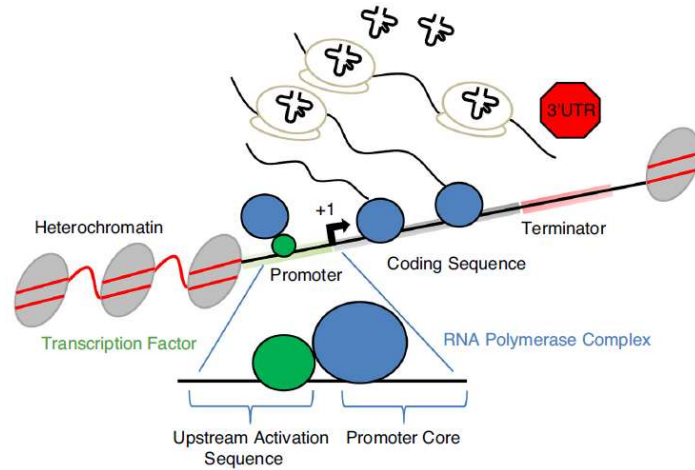


- Tuning Yeast promoters through synthetic transcription factors: fusion of **ZNF motif** and trans-activating **VP16** domain.
- Each ZNF domain is **30 aa** long and forms a $\beta\beta\alpha$ configuration, where α -helix AAs interact with **3 nts** in the major groove of DNA.

Tuning Output By ZF-DNA Affinity

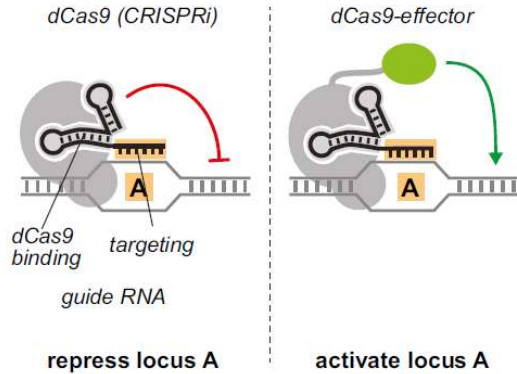


Computational design of Synthetic Yeast Promoters

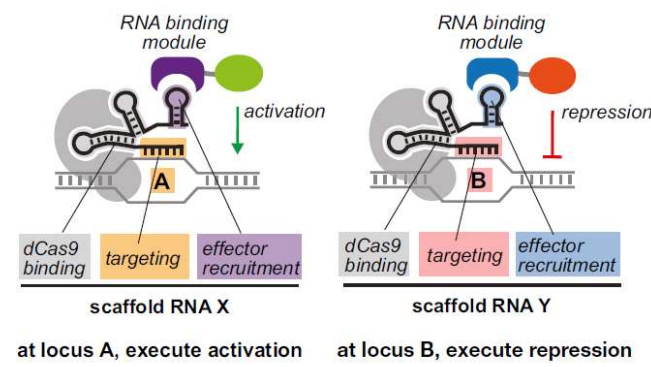


- Modulating nucleosome positioning allows synthetic design of Yeast promoters.

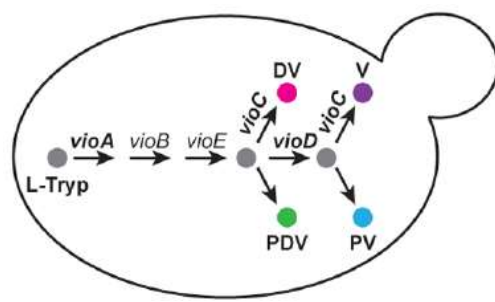
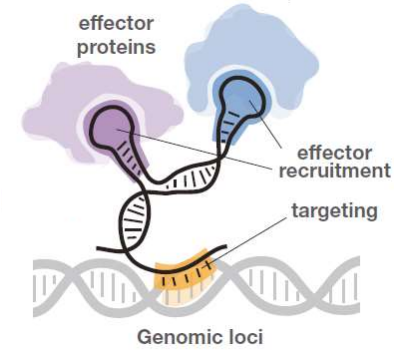
Tunable Yeast Promoters for Metabolic Engineering



guide RNA encodes locus only



scaffold RNA encodes locus and action



L-Tryp = L-tryptophan
PV = proviolacein
V = violacein
PDV = prodeoxyviolacein
DV = deoxyviolacein

Activation (<i>vioA</i>)	Activation (<i>vioC</i>)	Repression (<i>vioD</i>)	Major Product
-	-	-	-
↑	-	-	● PV
↑	↑	-	● V
↑	-	↓	● PDV
↑	↑	↓	● DV

- CRISPR/ dCas9 for programmable activation/ repression of metabolic genes

Eukaryotic translation

- Requires 5' cap (a guanine nucleotide connected to mRNA via an unusual 5' to 5' triphosphate linkage)
- IRESes can mimic the 5' cap

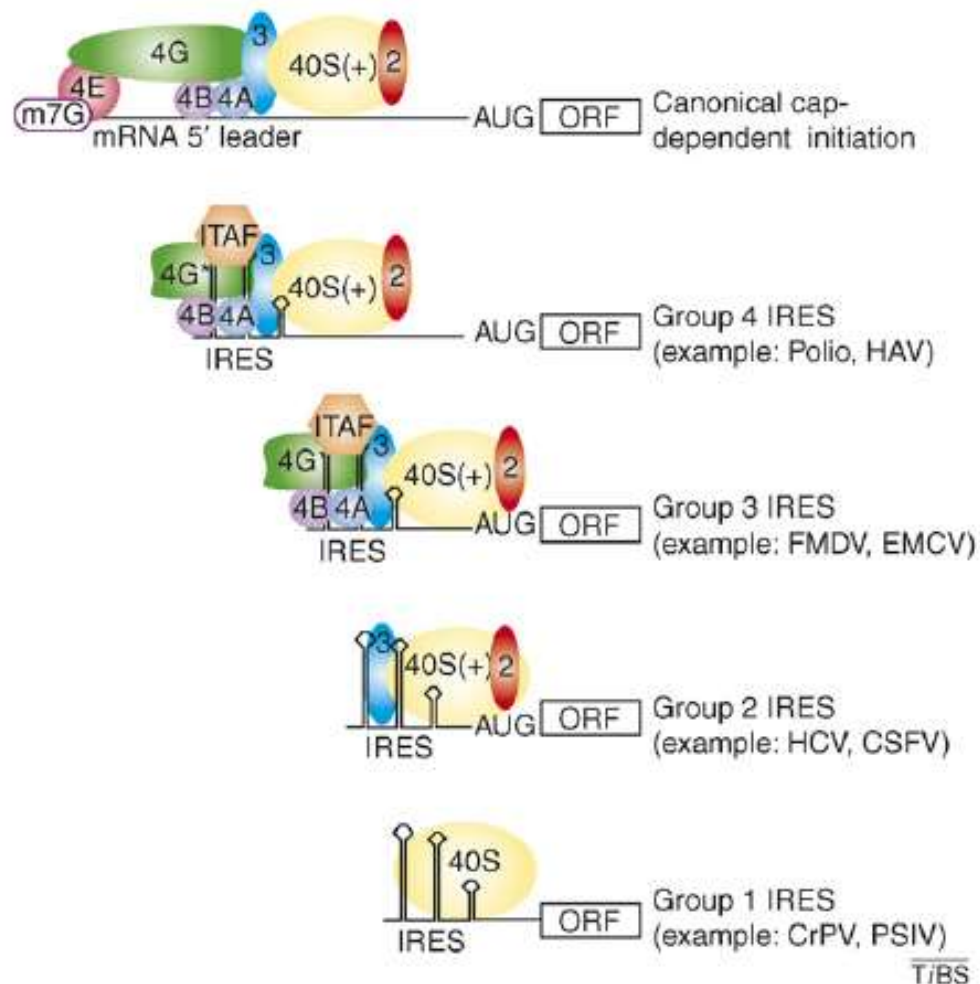
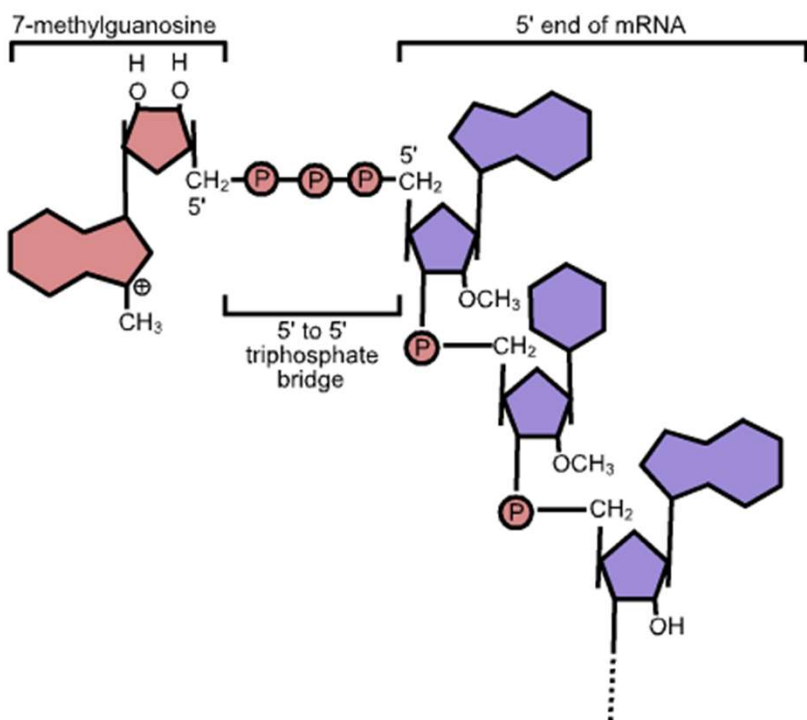


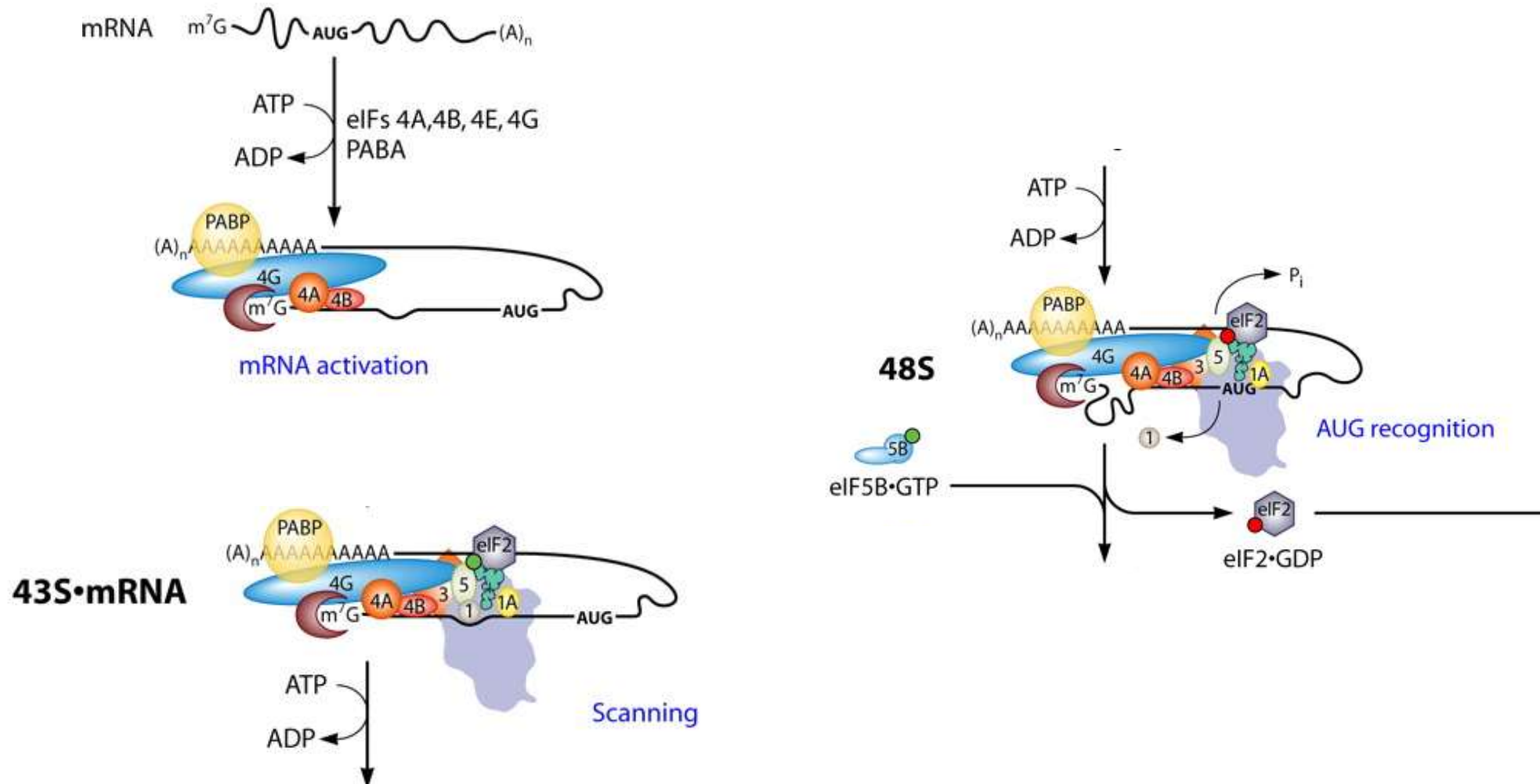
Image source/s:

https://en.wikipedia.org/wiki/Five-prime_cap

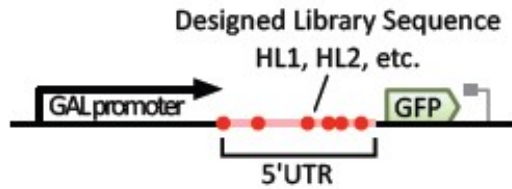
(Kieft J, 2008. Trends in Biochemical Sciences.)

Eukaryotic translation

- Ribosomal scanning mechanism

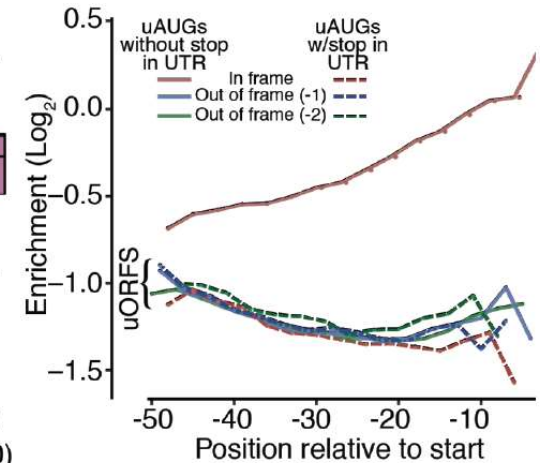
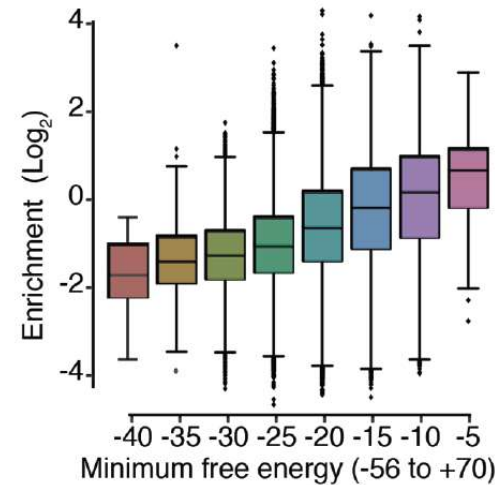
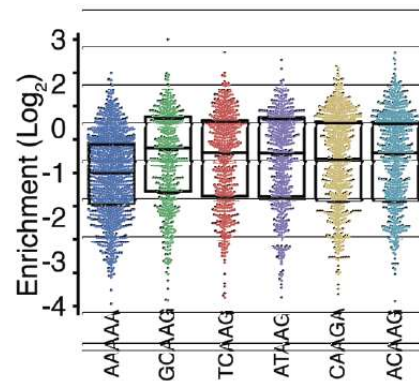
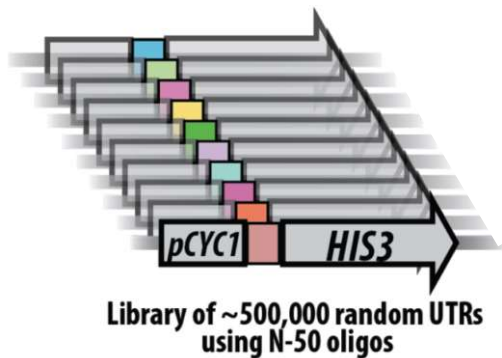
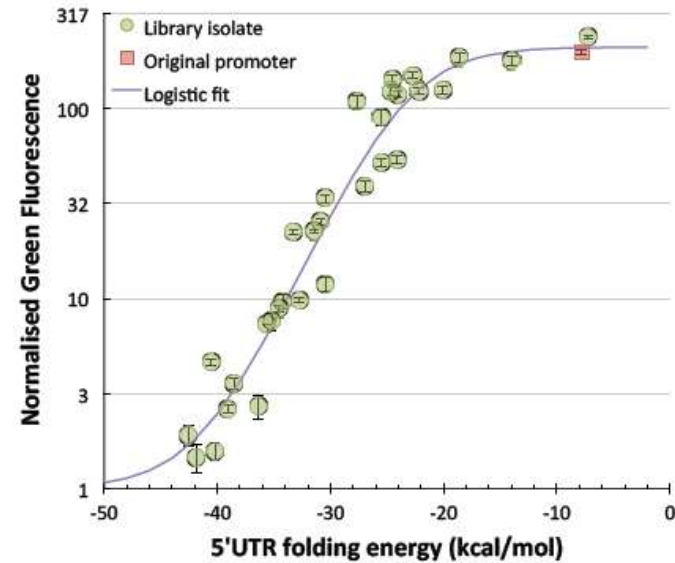


Translation Calculator in Eukaryotes?



$$Fl_n(\Delta G) = 1 + \frac{P_{max}}{1 + e^{-k(\Delta G - x_0)}}$$

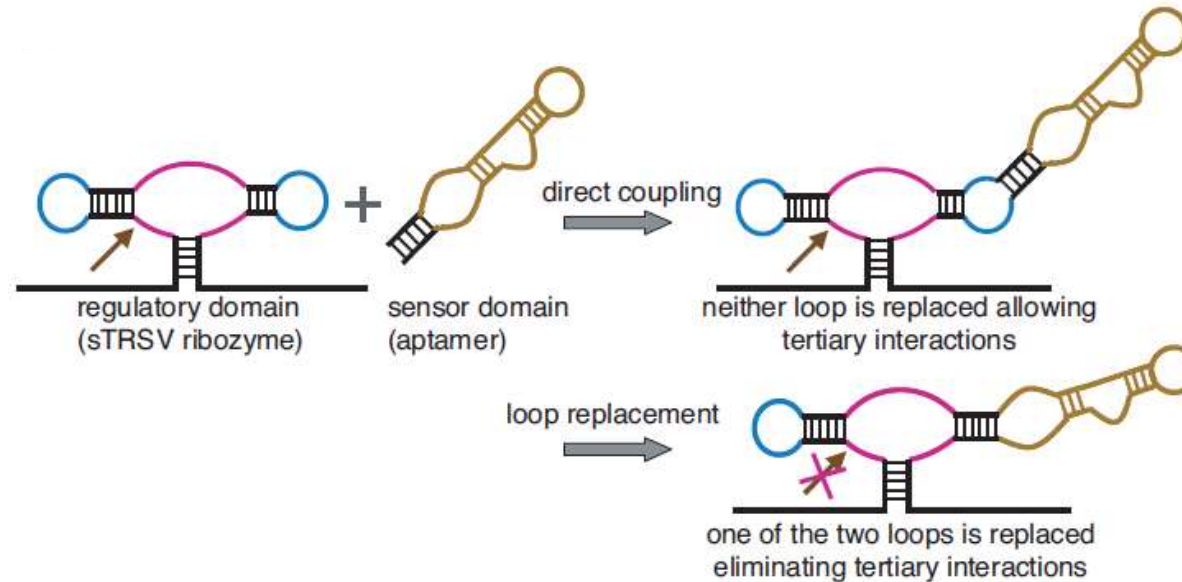
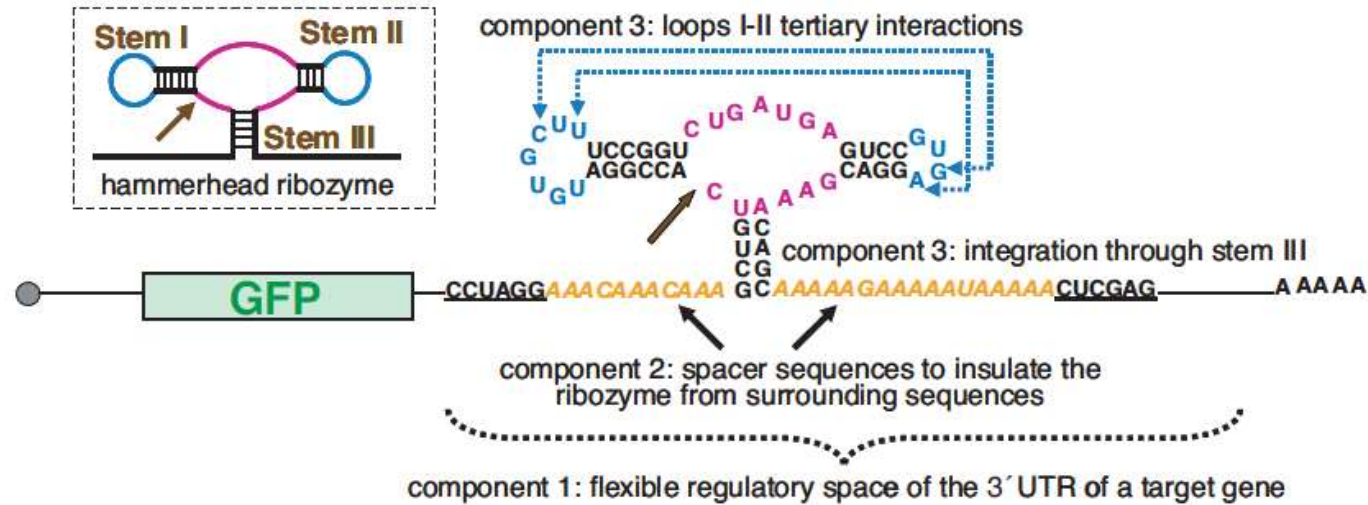
Fl_n = normalised fluorescence
 ΔG = minimum free energy of folding
 P_{max} = maximum promoter strength
 k = slope of the exponential section
 x_0 = location of the inflection point on a linear scale



- Modelling 5'UTR structure to predict mRNA translation rate in Yeast

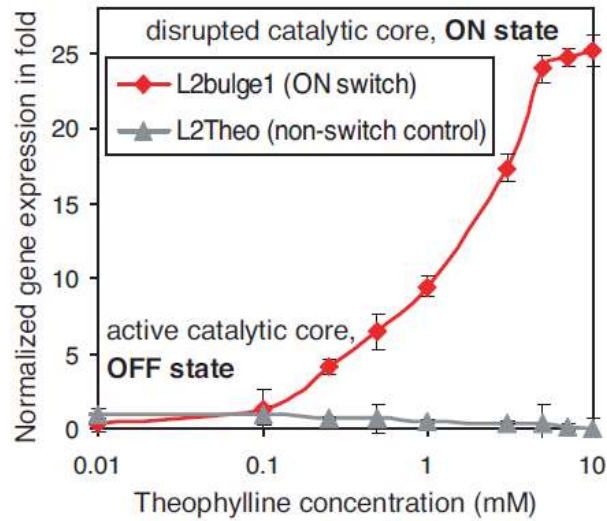
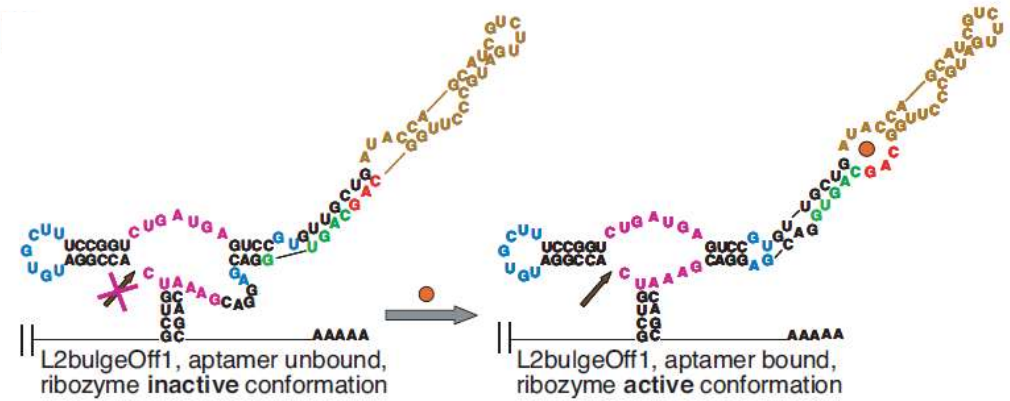
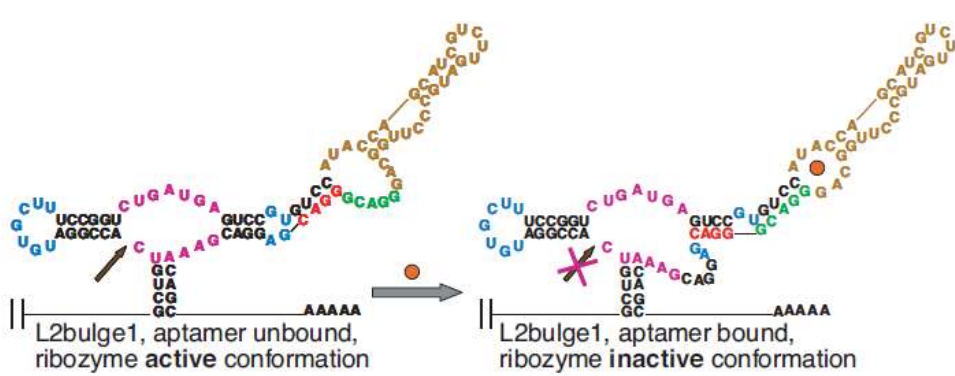
(Cuperus *et al.*, 2017. *Genome Research*.
 Weenink *et al.*, 2018. *Synthetic Biology*.)

Logic Gates using 3'UTR Riboswitches in Yeast

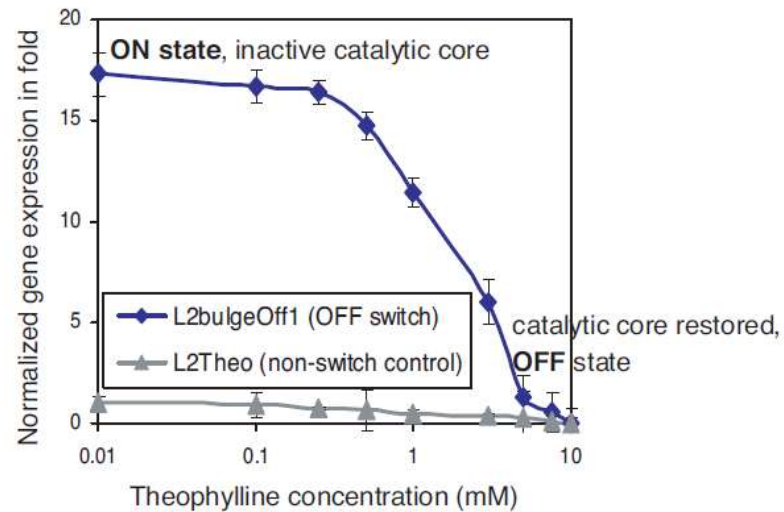


- Small-molecule activated hammerhead ribozyme alters mRNA stability

Logic Gates using 3'UTR Riboswitches in Yeast



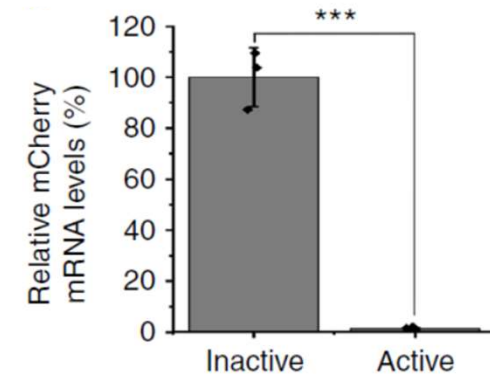
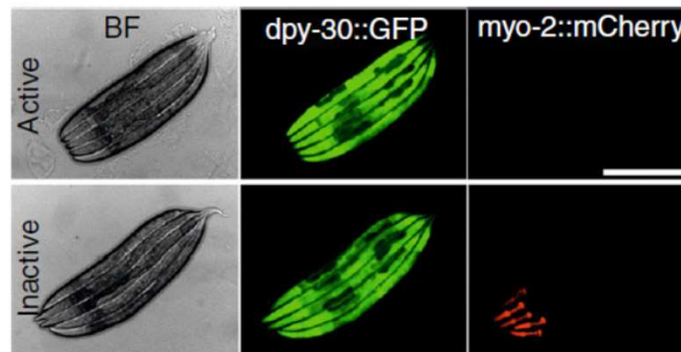
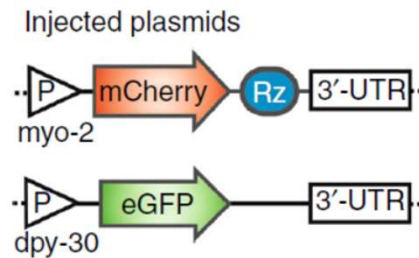
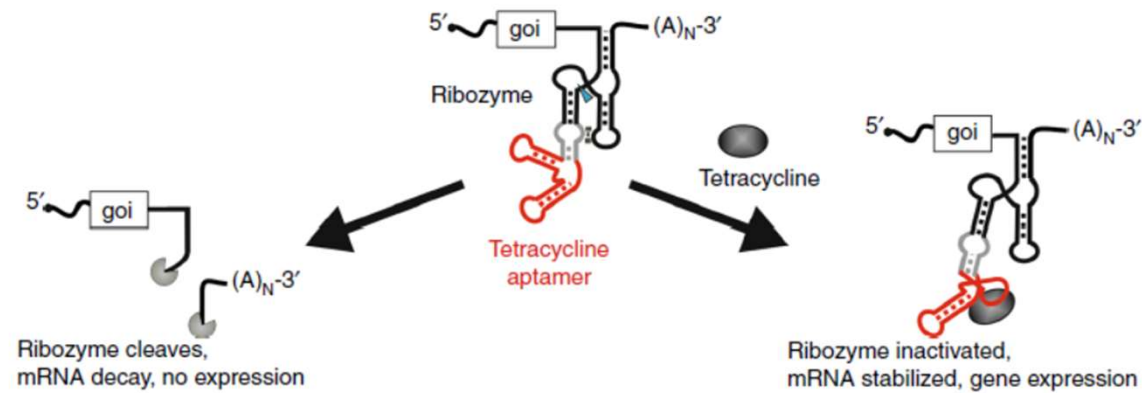
Theo-ON



Theo-OFF

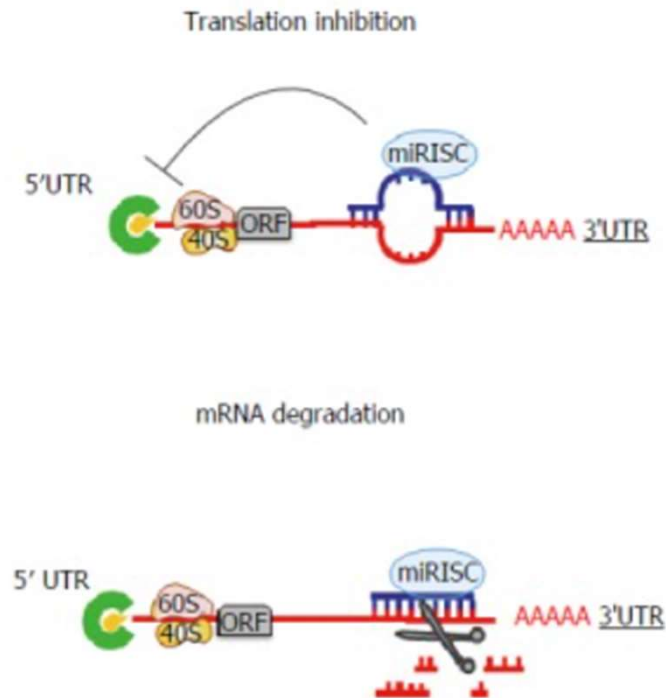
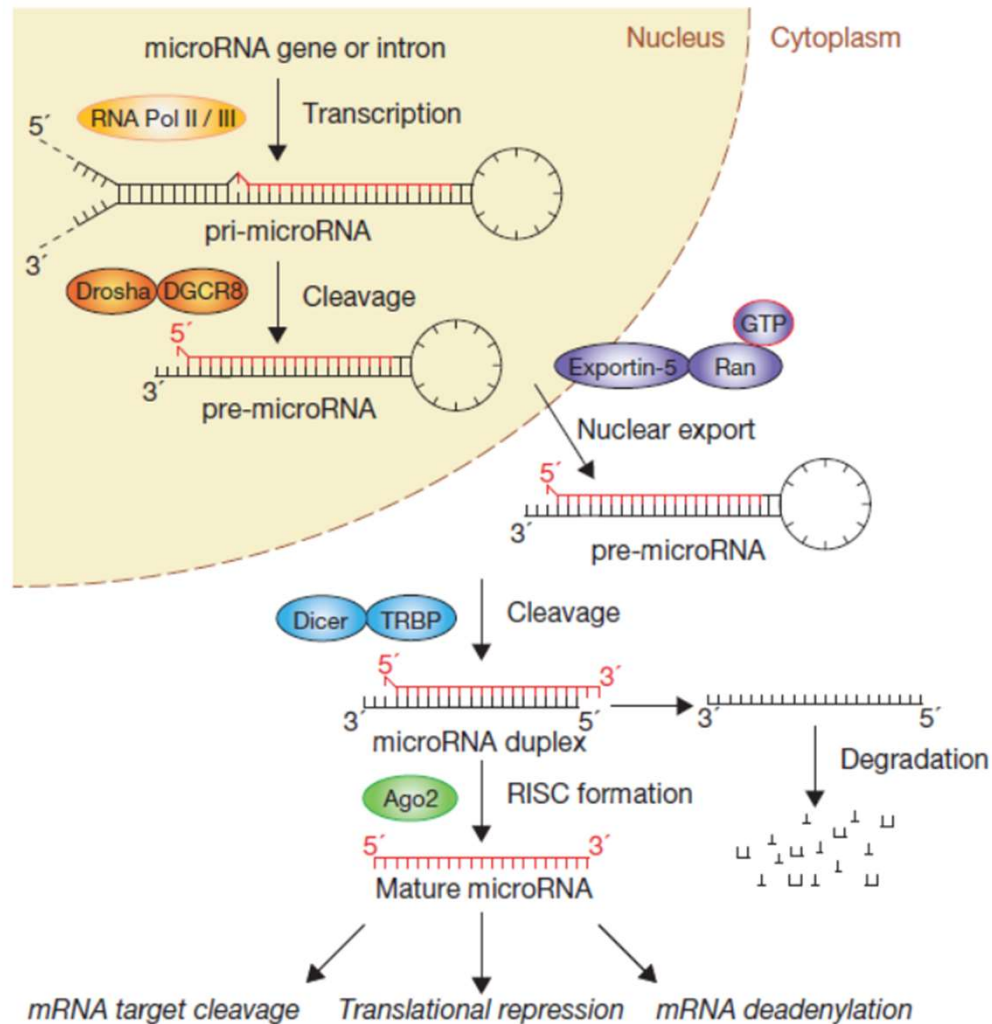
- Small-molecule activated hammerhead ribozyme alters mRNA stability

Logic Gates using 3'UTR Riboswitches in worms



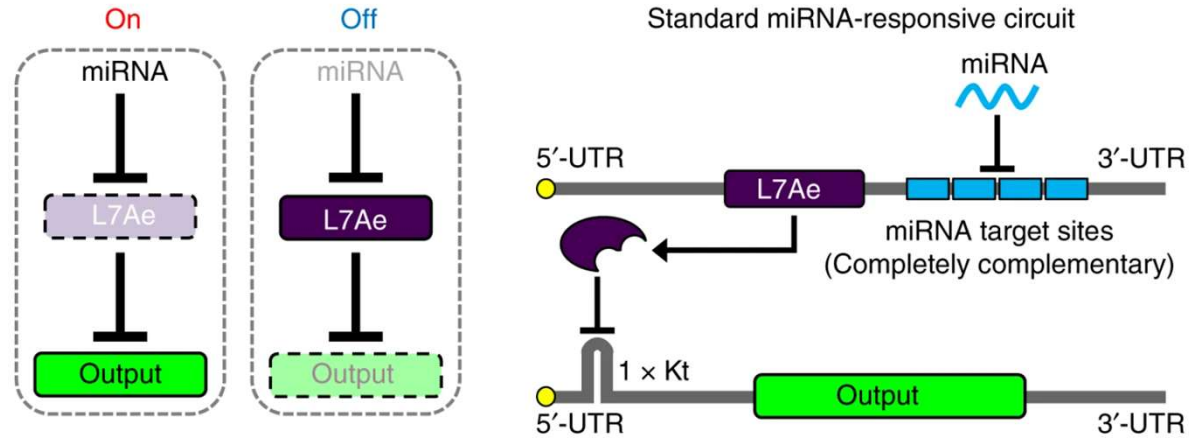
- Small-molecule activated hammerhead ribozyme alters mRNA stability

microRNAs for translation regulation



- microRNAs can emerge from diverse RNA sequences
- They can cause both translation inhibition and mRNA degradation

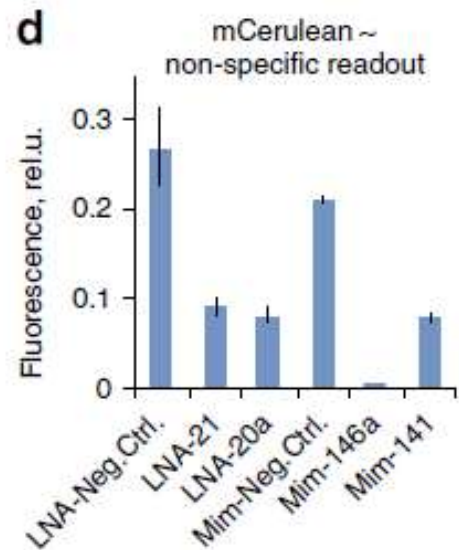
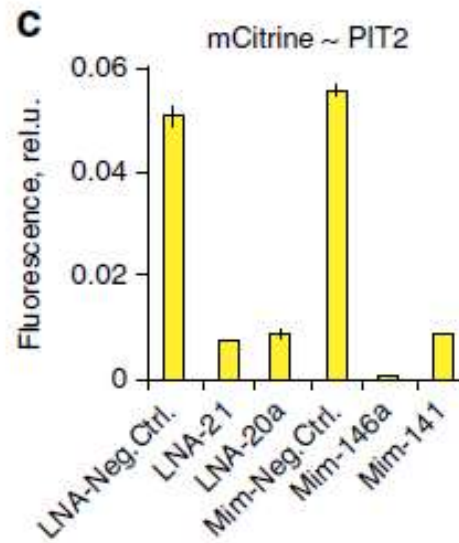
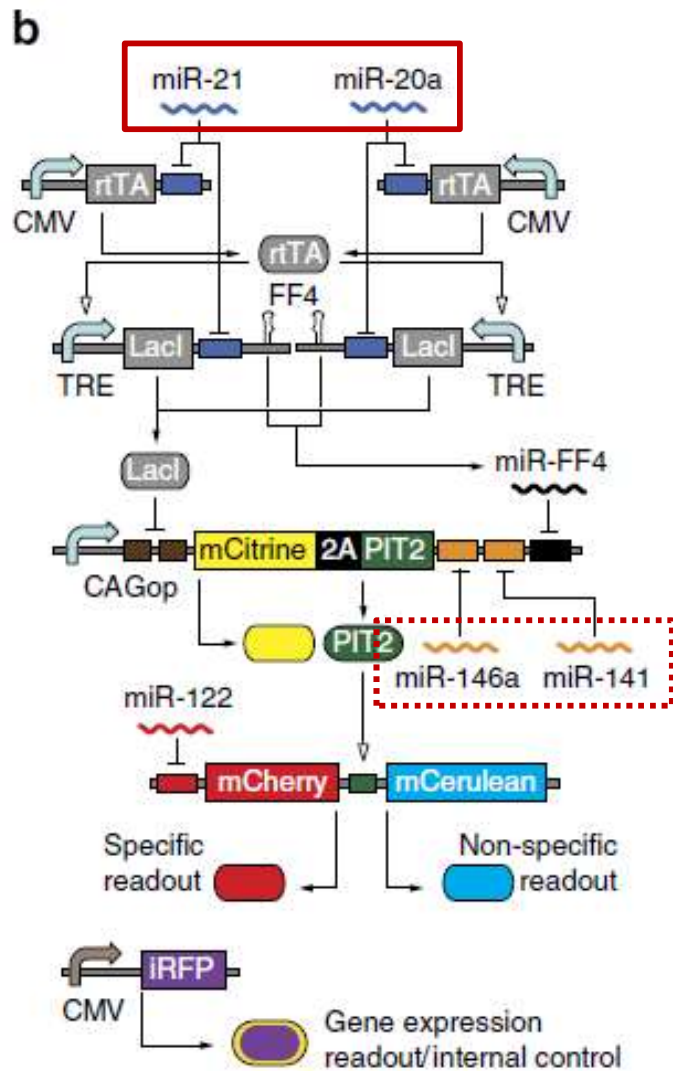
Synthetic RNA logic in Mammalian Cells



Gate	Truth table			Circuit design	Scatter plot	EGFP/iRFP670 [a.u]
AND	miR-21	miR-302a	Output			
	0	0	0			
	1	0	0			
	0	1	0			
OR	miR-21	miR-302a	Output			
	0	0	0			
	1	0	1			
	0	1	1			

- microRNA programming using RNA interaction logic

Programming RNA stability in Mammalian Cells



rtTA = reverse Tet transactivator

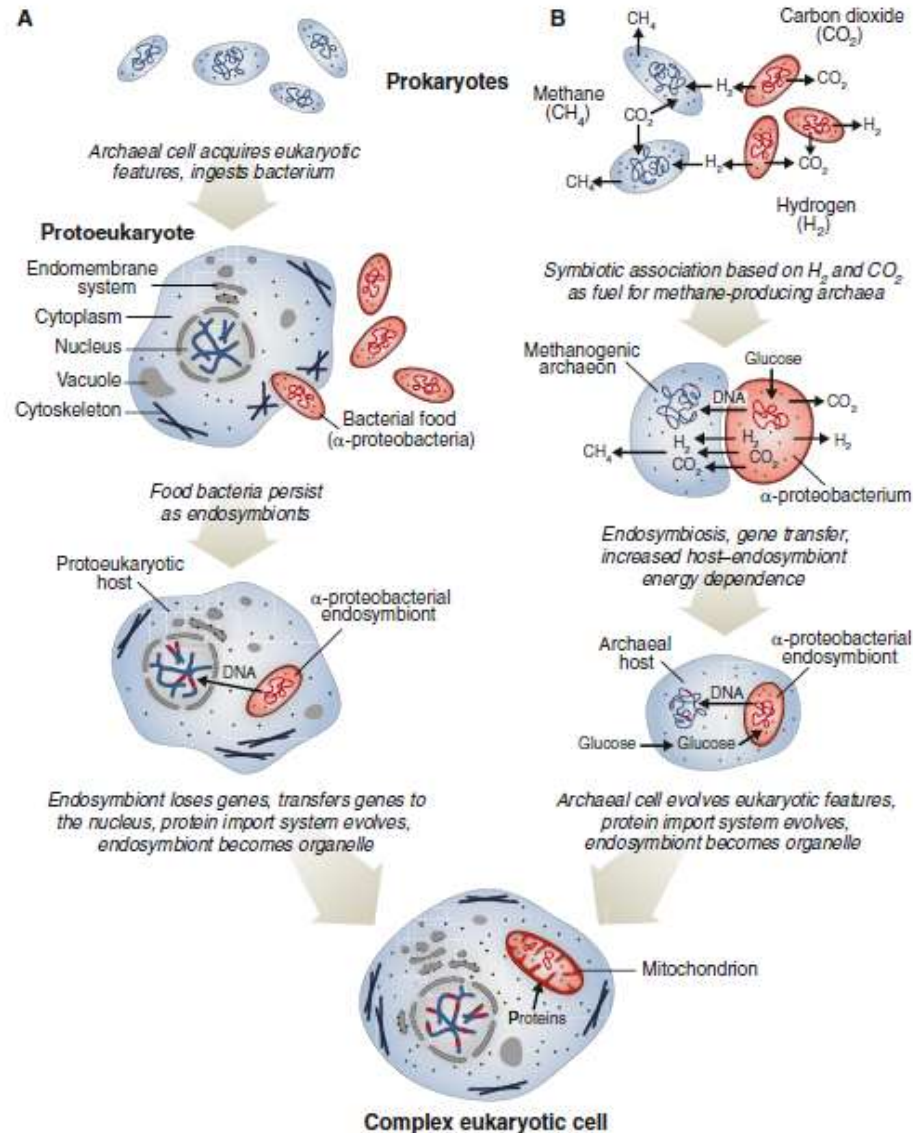
2A = self-cleaving peptide

LNA = locked nucleic acid

PIT2 = Streptogramin-responsive transactivator

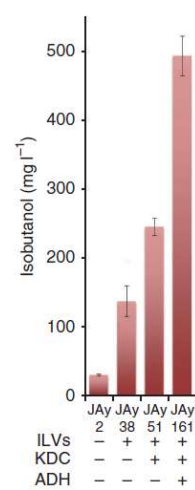
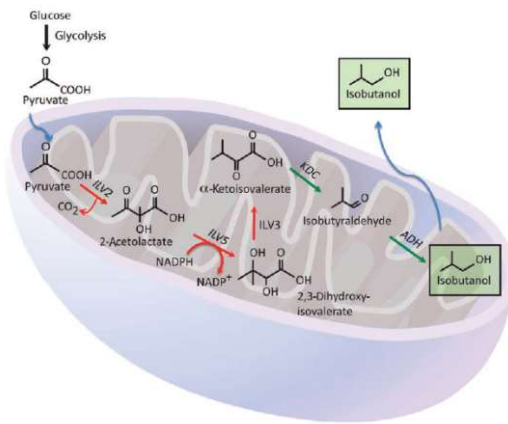
- microRNA programming using RNA interaction logic

Evolutionary history: Once upon a time Eukaryotes were Archaeobacteria

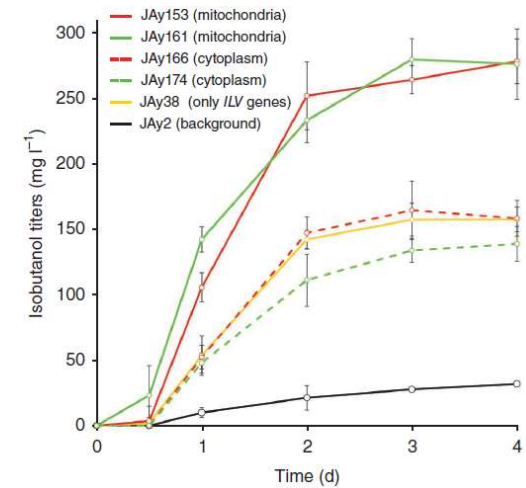
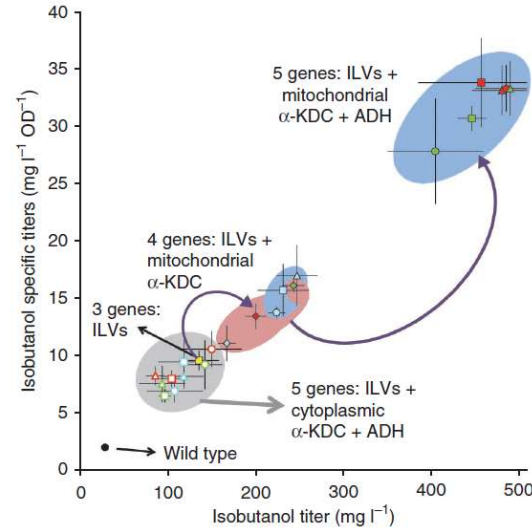


Current Biology

Simpler Compartments: mitochondrion and chloroplast



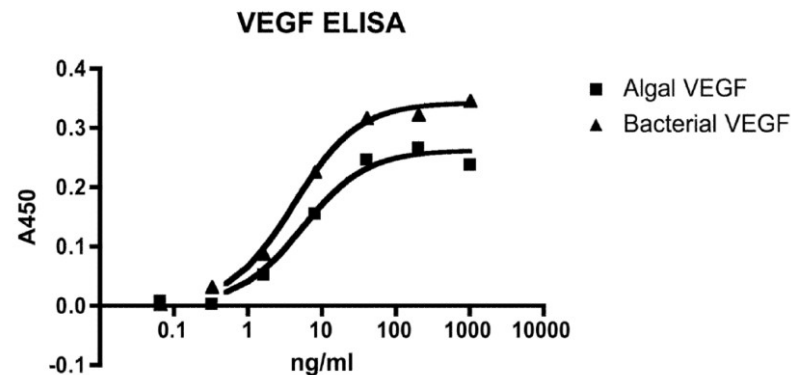
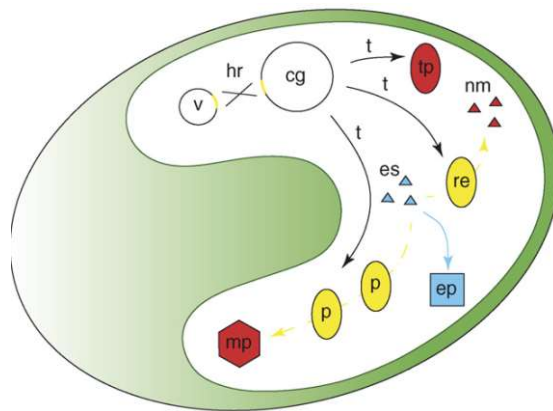
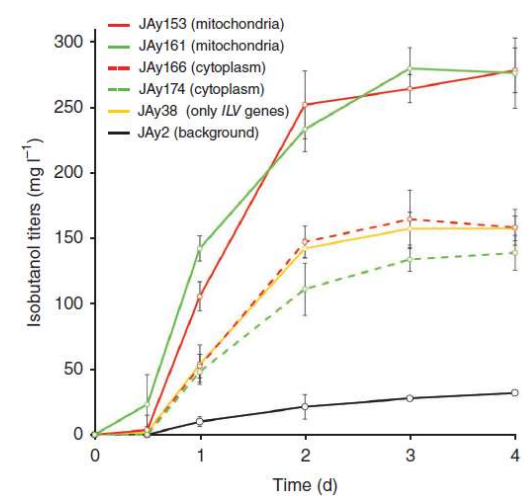
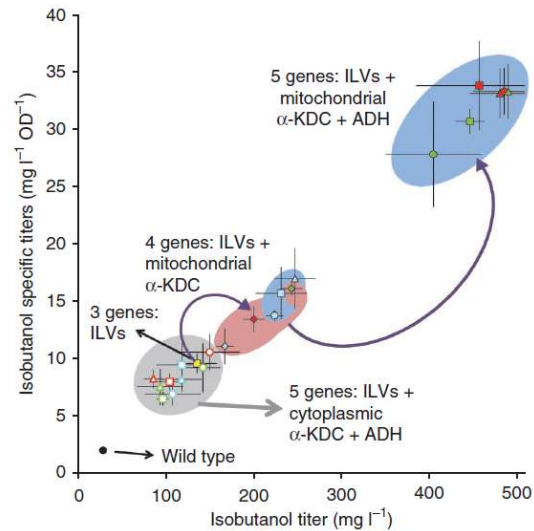
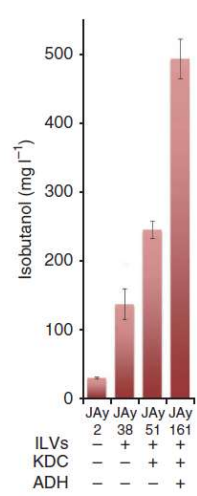
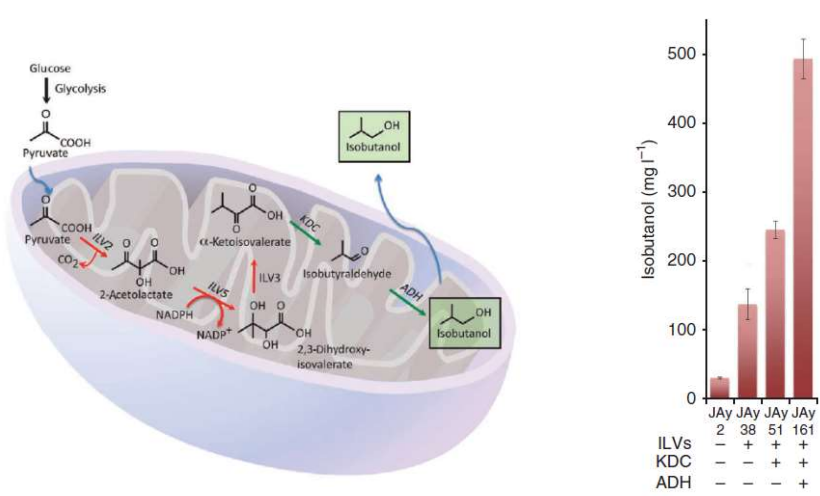
ILVs	KDC	ADH
-	-	-
+	-	-
+	+	-
+	+	+



- Eukaryotic organelles allow localised concentration of enzymes, and simpler expression

(Avalos *et al.*, 2013. *Nat. Biotech.*
 Mayfield *et al.*, 2007. *Curr. Op. Biotech.*
 Rasala *et al.*, 2010. *Plant Biotech. Journal*)

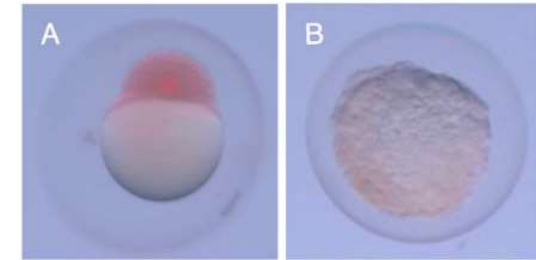
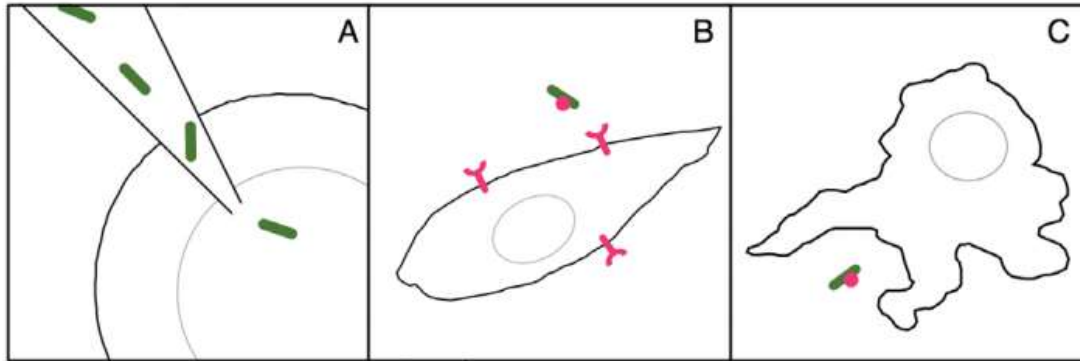
Simpler Compartments: mitochondrion and chloroplast



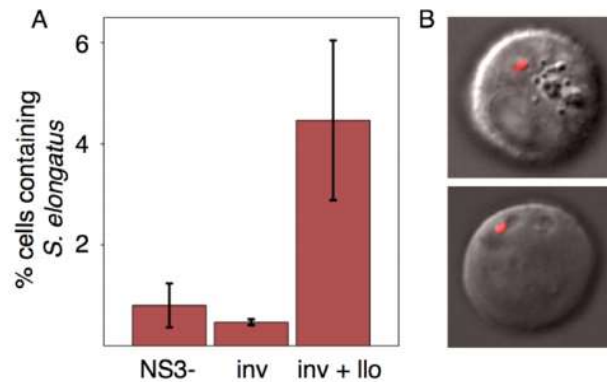
- Eukaryotic organelles allow localised concentration of enzymes, and simpler expression

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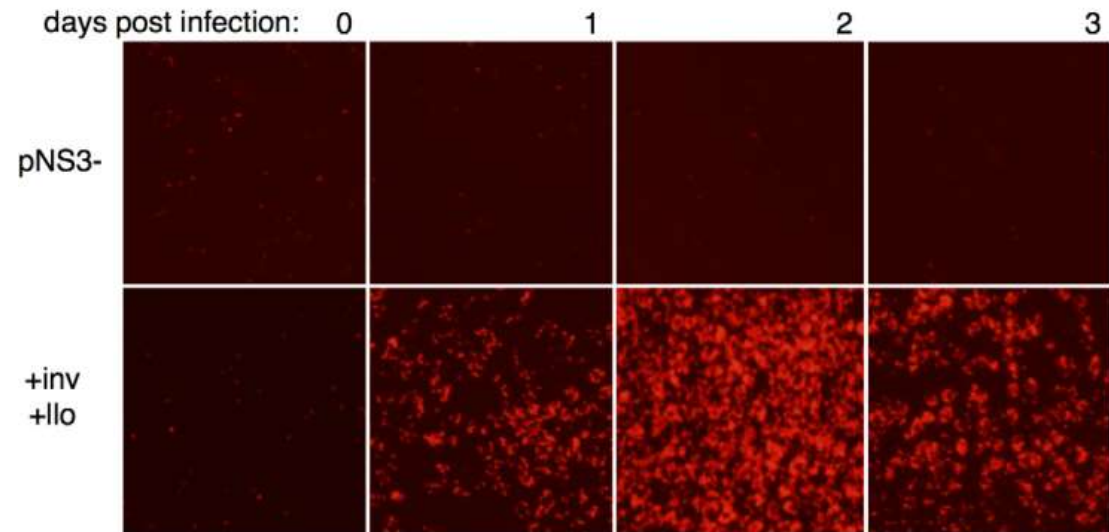
Building synthetic organelles



Zebrafish embryos
(*E. coli*)



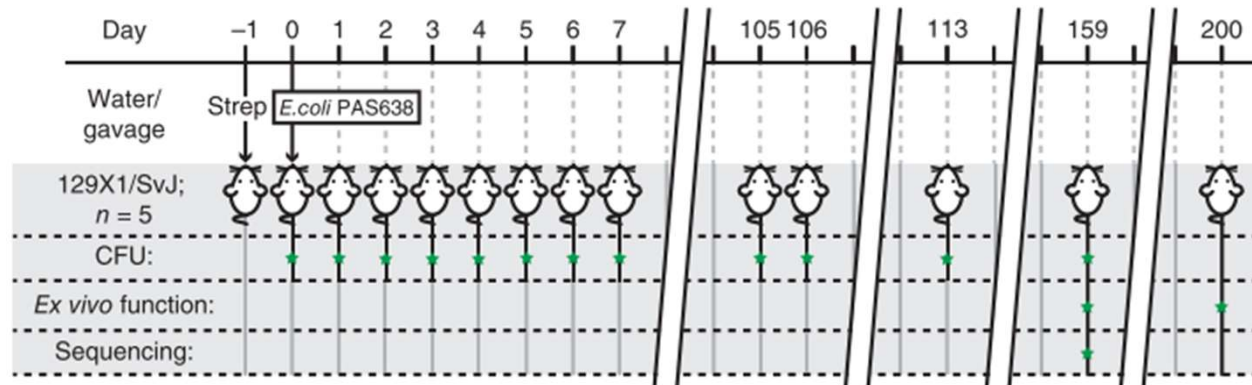
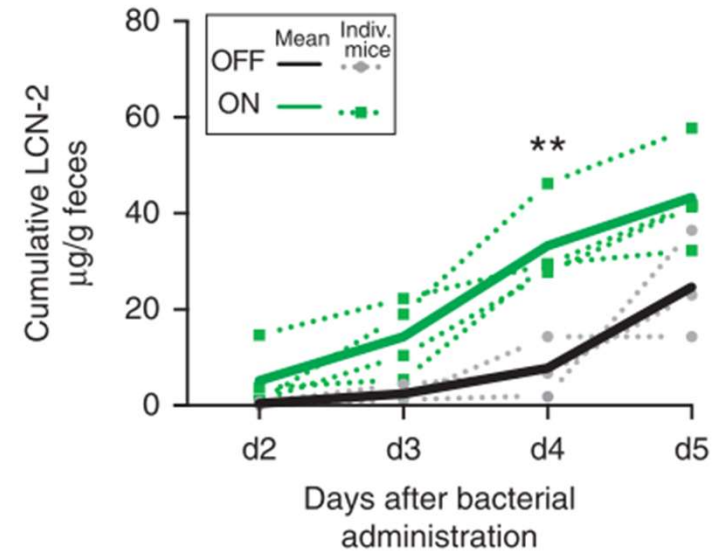
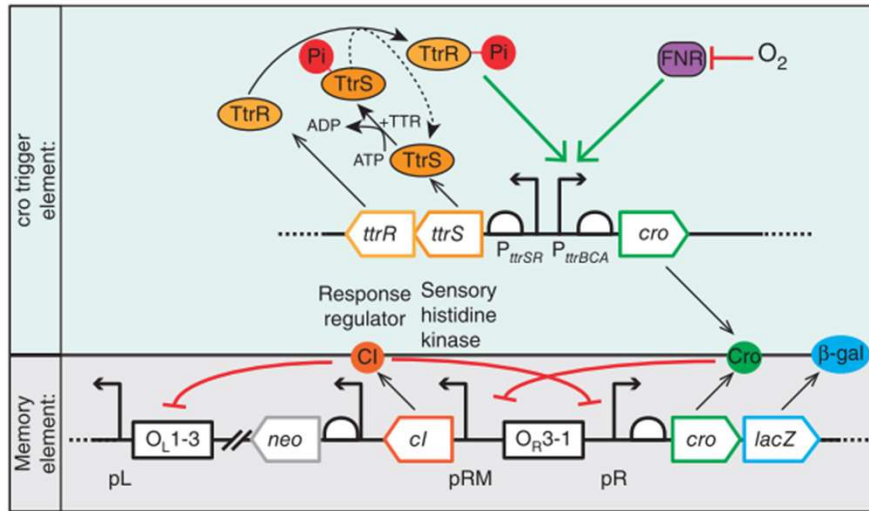
CHO cells
(*Synechococcus elongatus*)



J774 macrophage cells
(*Synechococcus elongatus*)

- Using bacteria to colonise mammalian cells in order to re-establish endosymbiosis

Sensor microbiome in mammalian environments

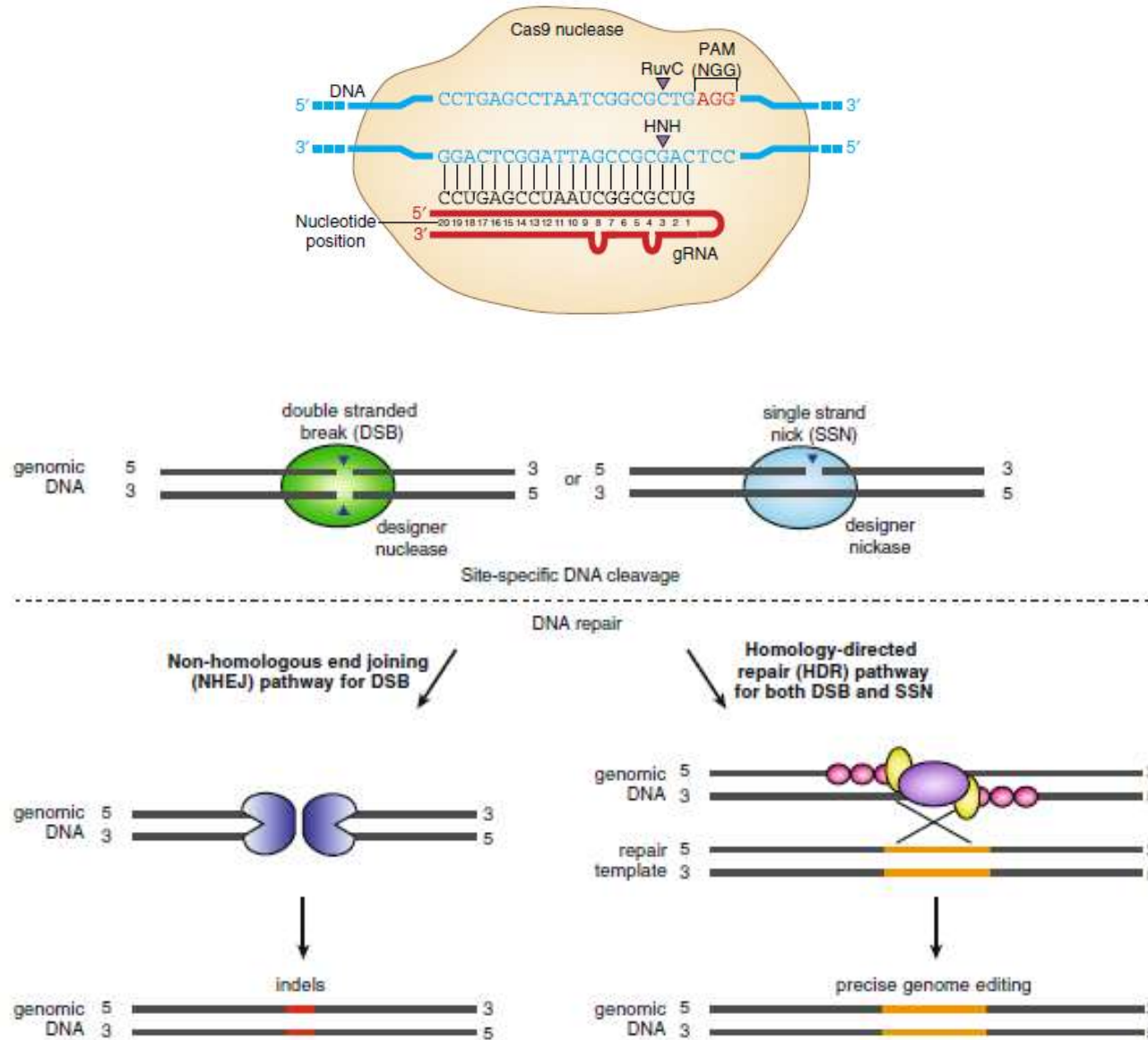


- In the presence of **tetrathionate** (inflammation marker: *Salmonella* and *Yersinia* infection), **TtrS sensor** becomes phosphorylated, in turn phosphorylating **TtrR activator**, which activates expression through P_{trrBCA} in anaerobic conditions.

(Riglar *et al.*, 2017. Nat. Biotech.)

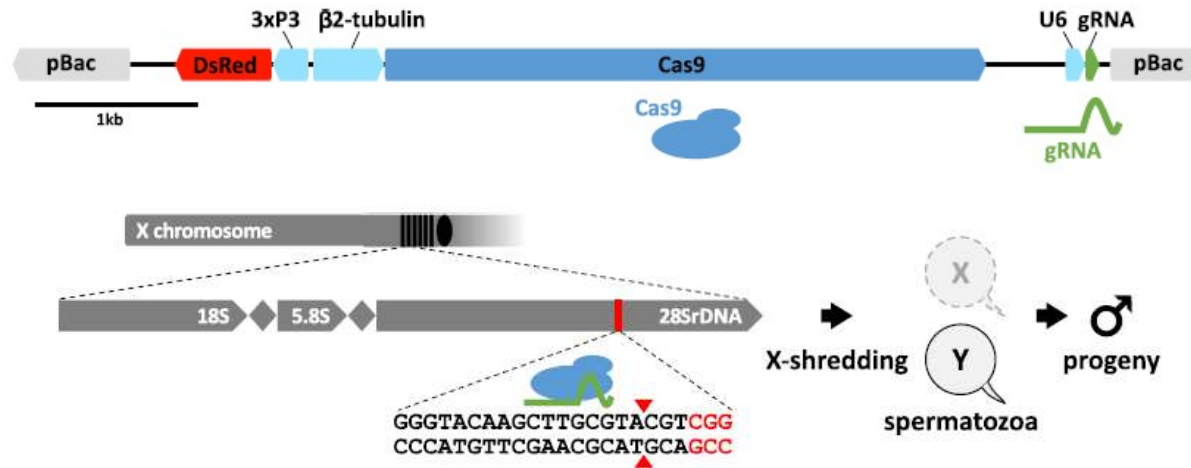
Genome Engineering with CRISPR

- A programmable CRISPR nuclease can be used to cause a double strand break at a precise location identified by its 20nt “guide RNA”

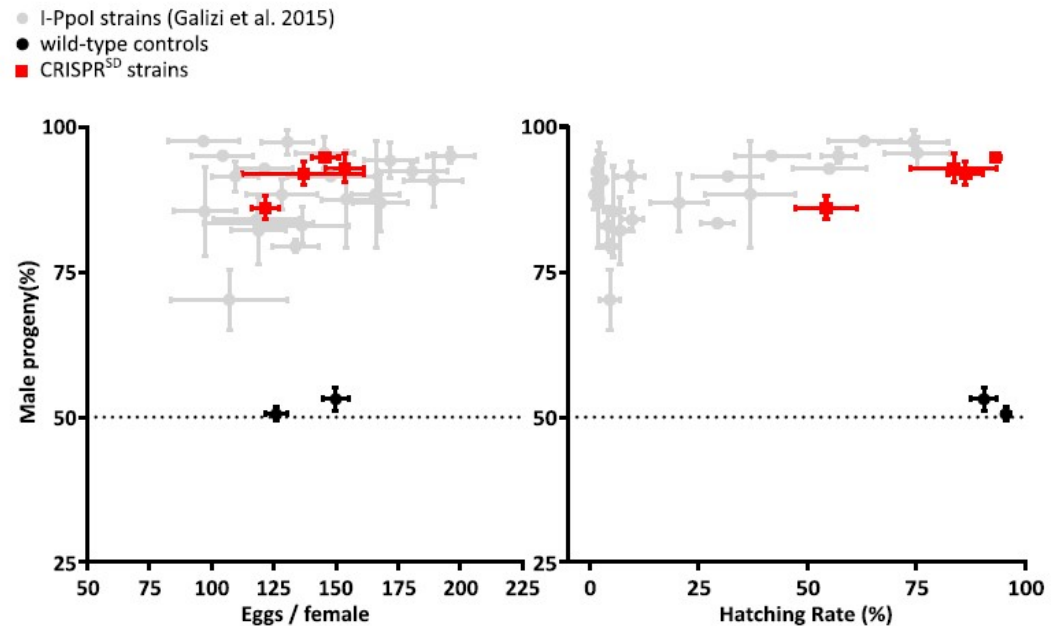


(Sander & Joung, 2014. Nat. Biotech.
Cong & Zhang, 2015. Methods in Mol Bio)

Skewing mosquito sex ratio

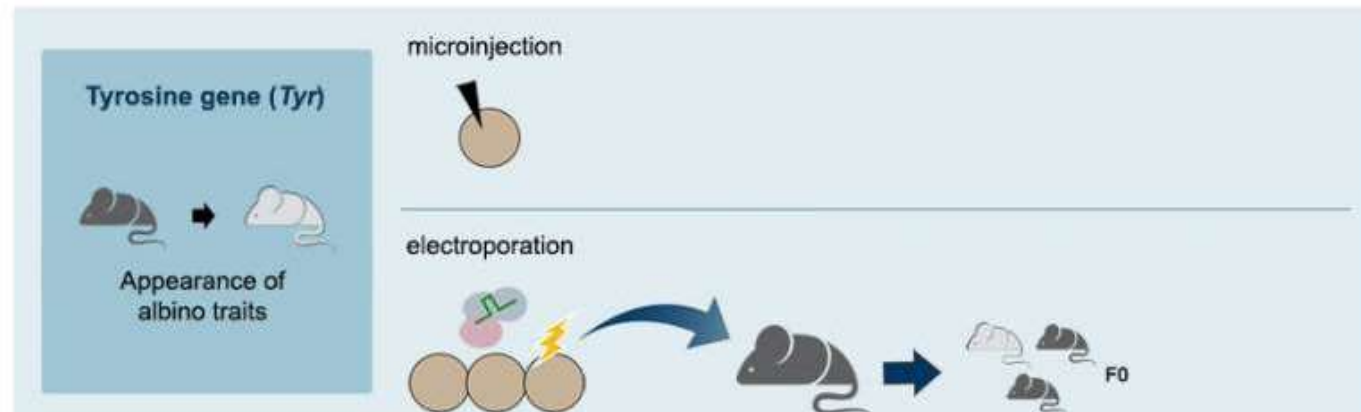
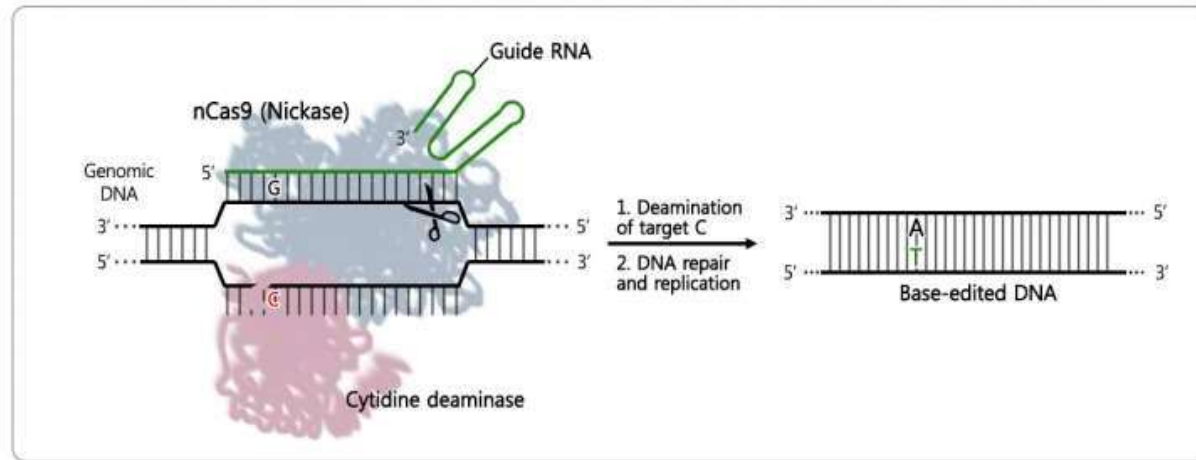


- Nuclease activity (I-PpoI/ Cas9) was used to target rDNA locus on the X-chromosome



(Galizi et al., 2014. Nat. Commun.
 Galizi & Hammond et al., 2016. Sci. Reports)

Base editing using CRISPR targeting in mouse embryos



- By fusing a base-editing cytidine deaminase to Cas9, specific nucleotides in the genome can be edited
- Changing a single nucleotide in the **dystrophin gene (Dmd)** or the **tyrosinase gene (Tyr)**. They were successful in both cases: Embryos with the single nucleotide mutation in the Dmd gene led to mice producing no dystrophin protein in their muscles, and mice with the Tyr mutation showed albino traits.

(Kim *et al.*, 2017. *Nat. Biotech.* 35, 435–437)

“Prime” editing using CRISPR nickase Cas9

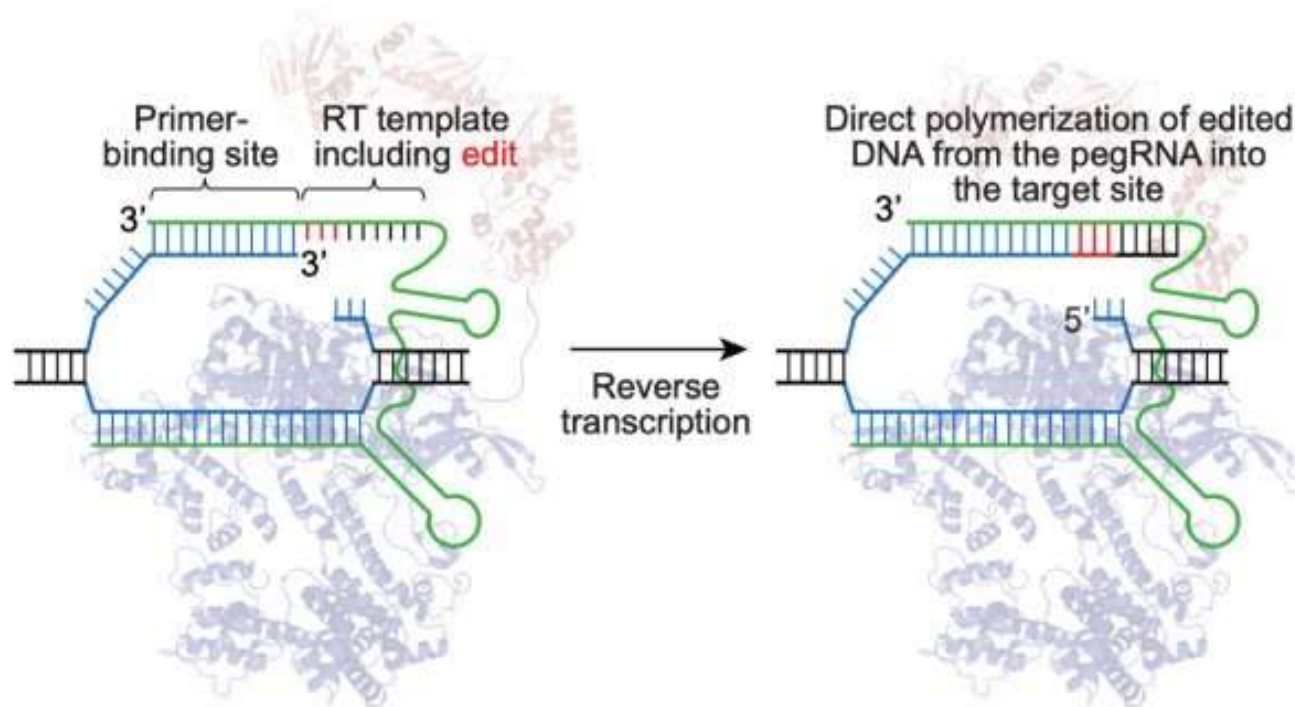


Figure 2: The prime editor with the pegRNA. The Cas9 portion of the editor cuts the genomic DNA and the reverse transcriptase portion polymerizes DNA onto the nicked strand based on the pegRNA sequence. Image from David Liu with permission.

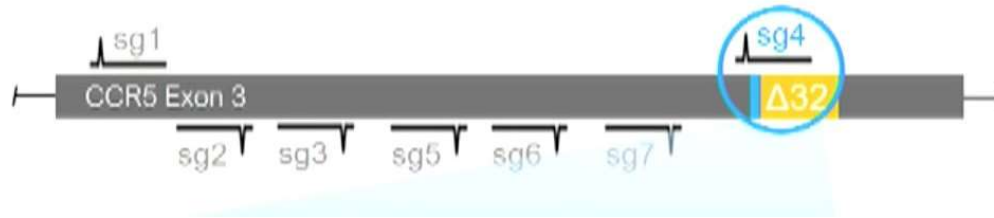
- By fusing a reverse transcriptase to **nickase Cas9**, a small number of specific nucleotides in the genome can be edited
- “They used CRISPR prime to perform more than 175 edits in human cells including targeted insertions, deletions, and all 12 types of point mutation”

(Anzalone *et al.*, 2019. Nature.)

<https://blog.addgene.org/prime-editing-crisp-cas-reverse-transcriptase>

CRISPR babies: How far have we come, and should we have?

- He Jiankui claimed during the Human Genome Editing Conference in Hong Kong in 2018 that he has made the world's first gene-edited babies

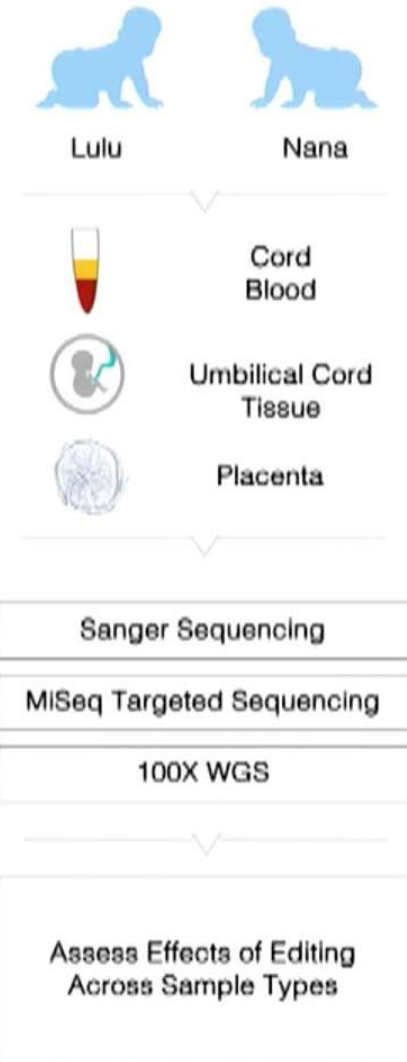


- *CCR5* codes for the HIV receptor on cell surface



Video stream of his talk:

<https://livestream.com/accounts/7036396/events/8464254/videos/184103056>



Questions welcome.

manish.kushwaha@inrae.fr