



# *Introduction to Gene Expression*



**Bayer Russia Biotechnology  
Conference**

**July 2023**





# My background in gene expression research

- // I have been active in gene expression research for 20 years:
  - // In *Drosophila*, mammalian, and plant systems
  - // Co-author on 10 publications in the scientific literature, including a commentary in *Cell*
  - // Inventor on 2 awarded patents and 3 additional published patent applications
- // I currently lead a molecular biology team and am a “go-to” person for gene expression troubleshooting at Bayer Crop Science



# Introduction to Gene Expression

## // Questions:

- // Why do we need to learn from plant cells for gene expression?
- // What is our current understanding of gene expression in plant cells?
- // How do we make use of this understanding to regulate transgene expression?

## // Topics:

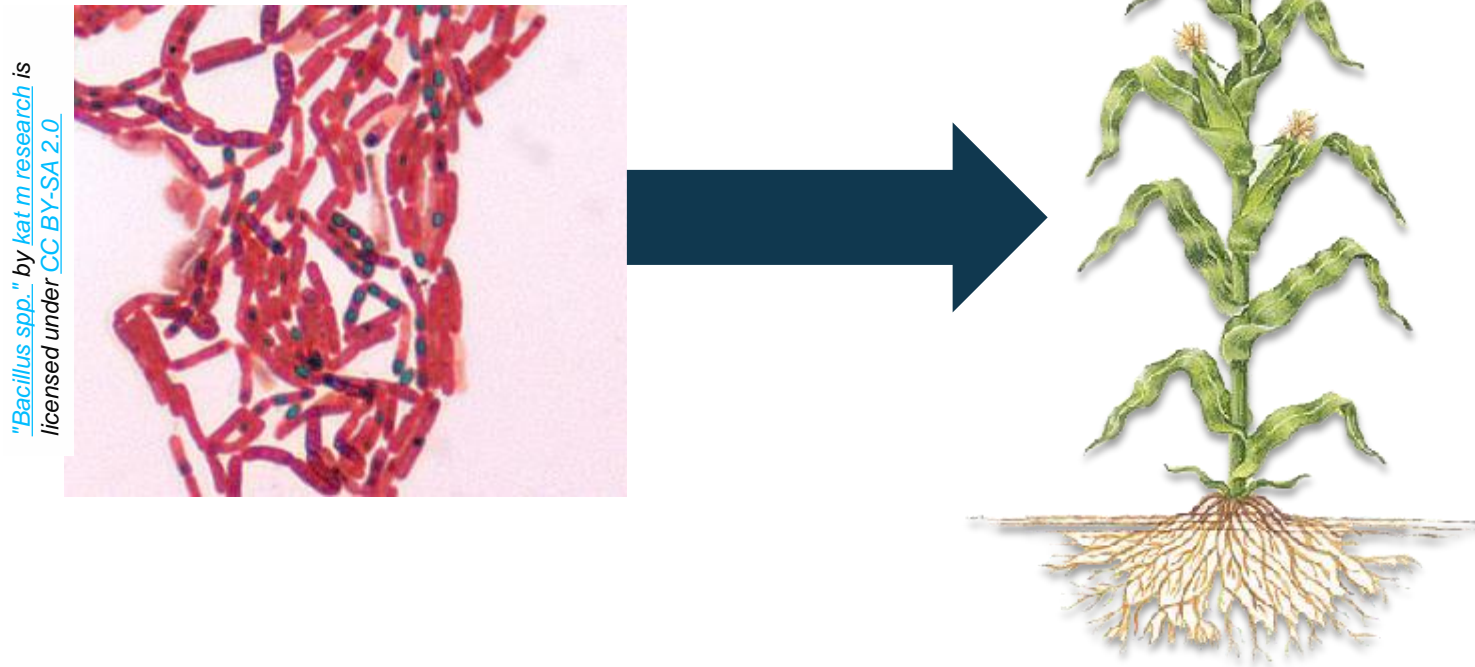
- // Chromatin, enhancers, and promoters
- // Co-transcriptional: splicing, polyadenylation and cleavage
- // Translation, codon optimization, and RNA stability
- // Vector Stacking

## // Themes:

- // Genes are expressed via structured complexes of nucleic acids and proteins
- // Gene expression processes are coupled



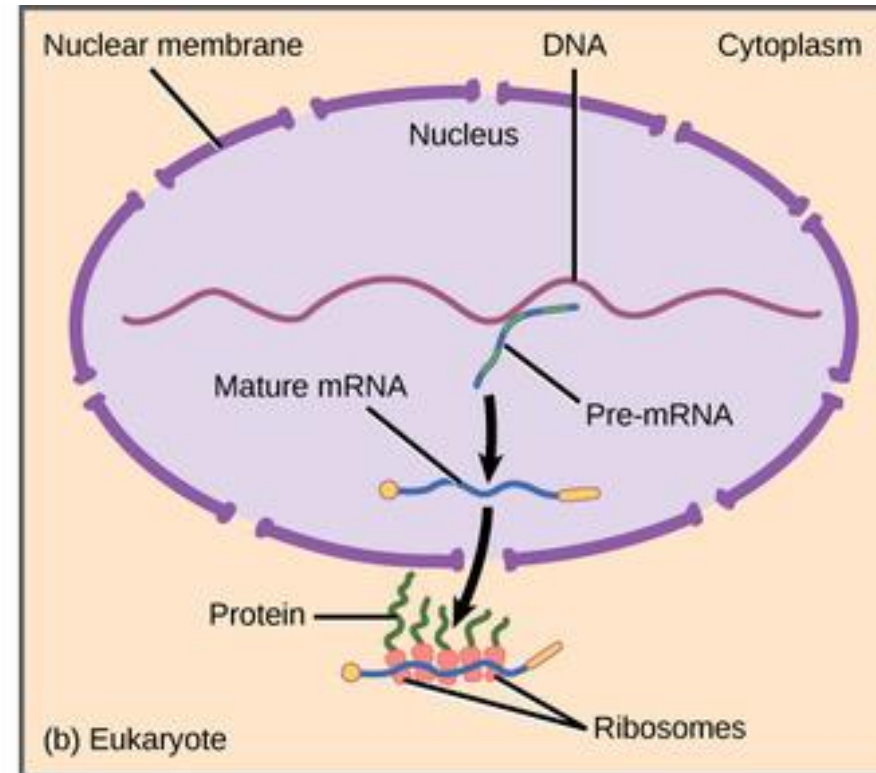
We often use bacterial genes to confer biotech traits, such as insect control



Diverged more than 2 billion years ago



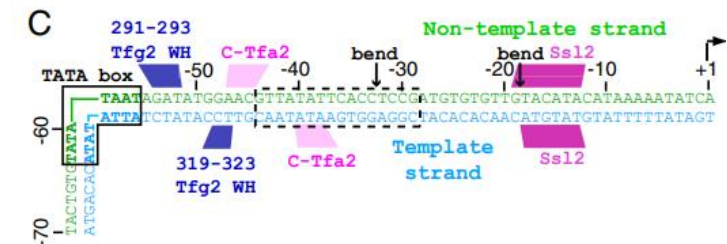
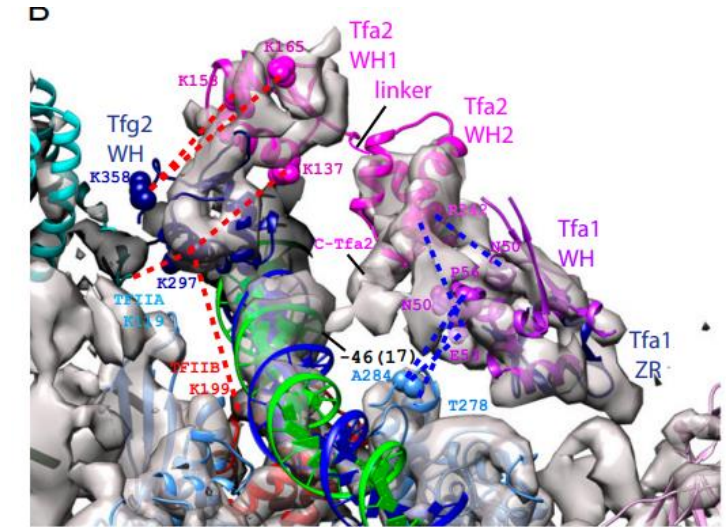
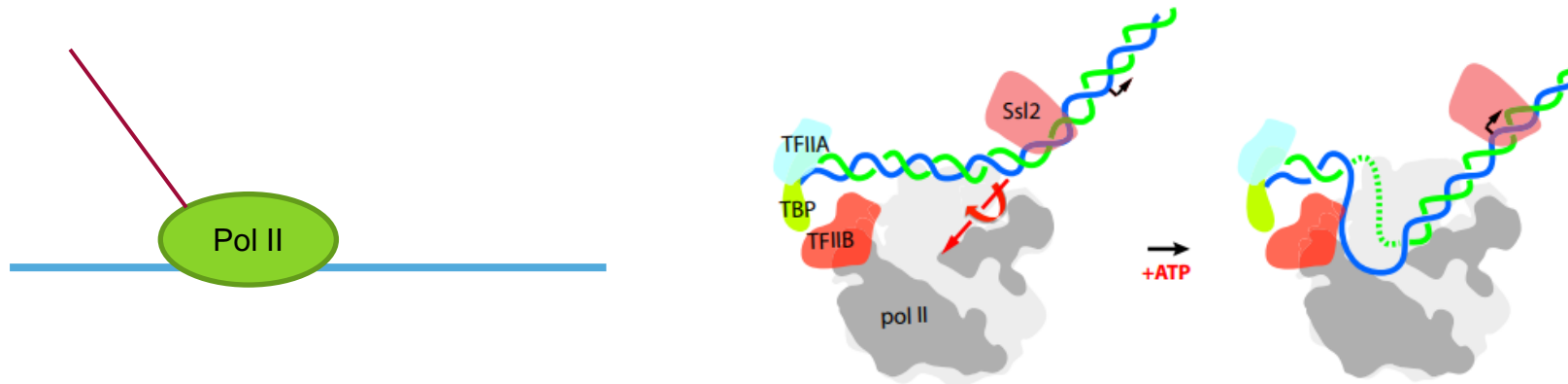
# There are important biological differences between bacterial and eukaryotic gene expression



([Libretexts.org](https://libretexts.org))



## Let's use models to learn, but keep in mind their limitations



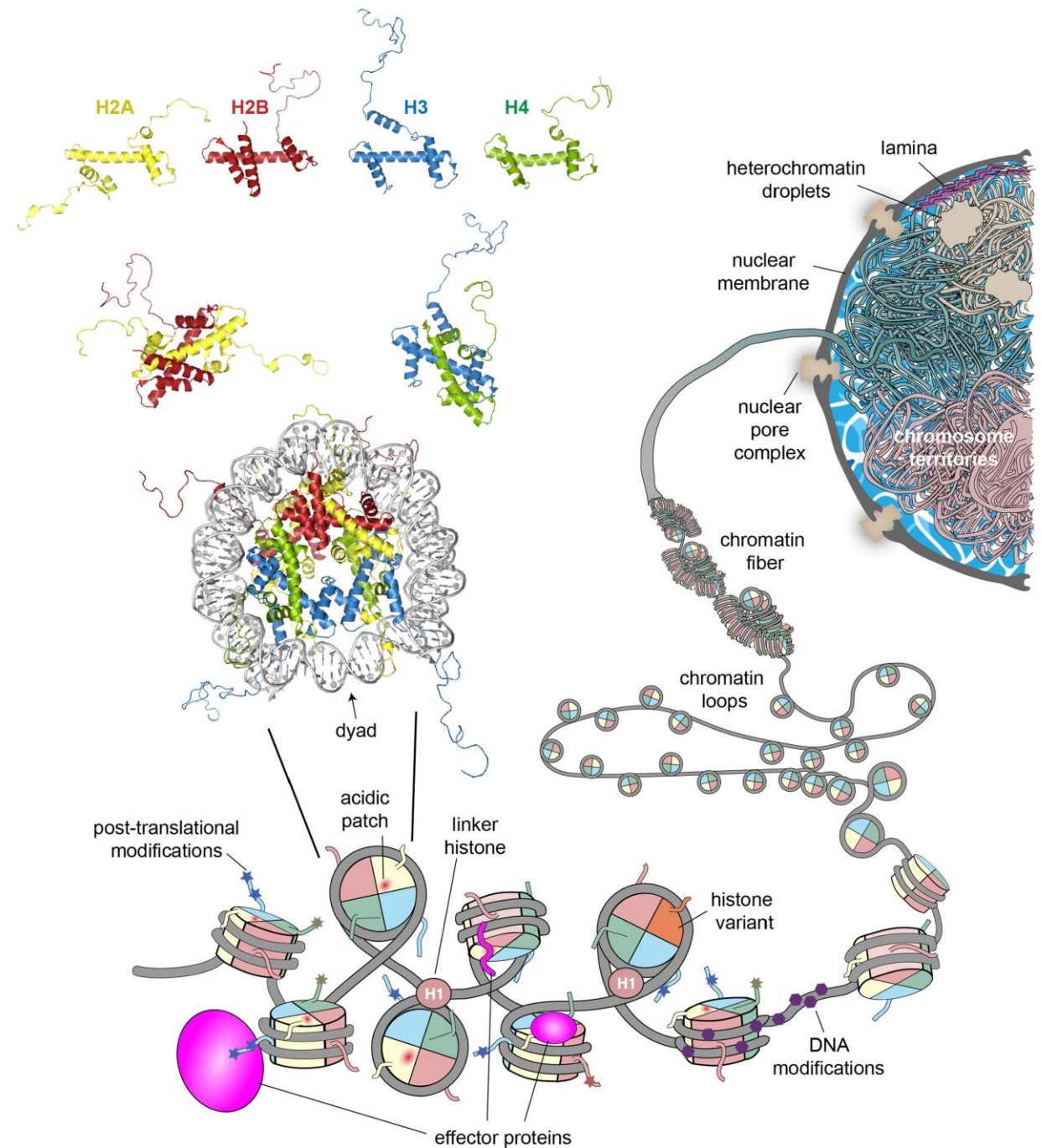
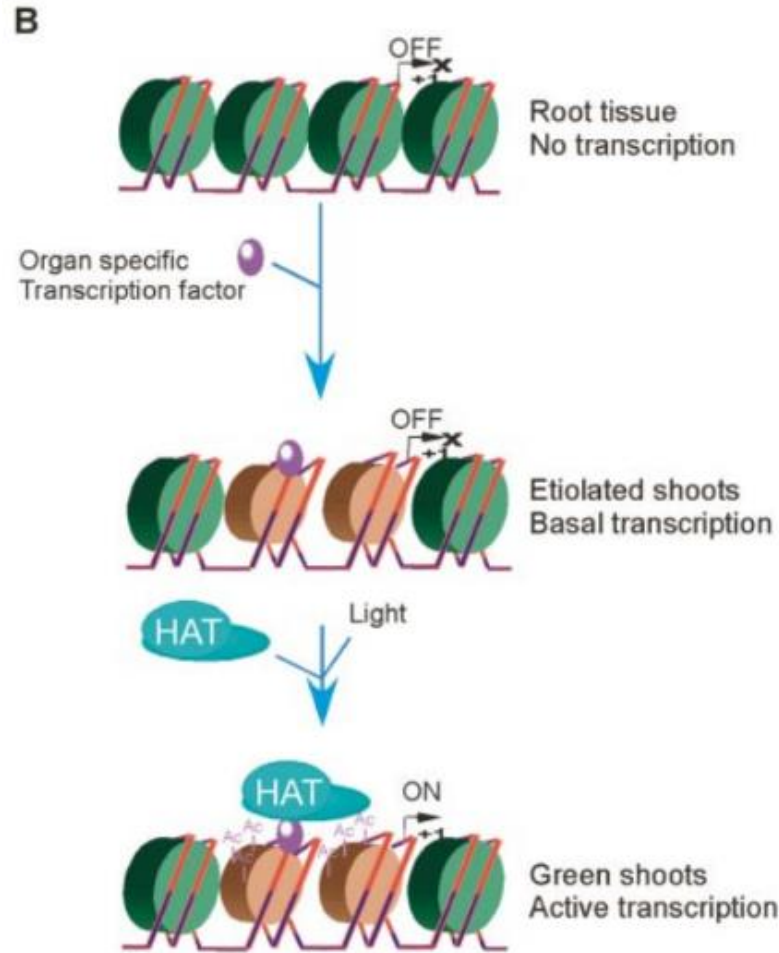
- Genes are expressed via nucleoprotein complexes

(Murakami et al, 2015. PNAS.)

# *Chromatin, enhancers, and promoters*

# Genes are made of DNA, and nuclear DNA is organized in chromatin

(Li et al, 2002. Bioessays.)



(Emmerik and Ingen, 2019. Progress in NMR.)

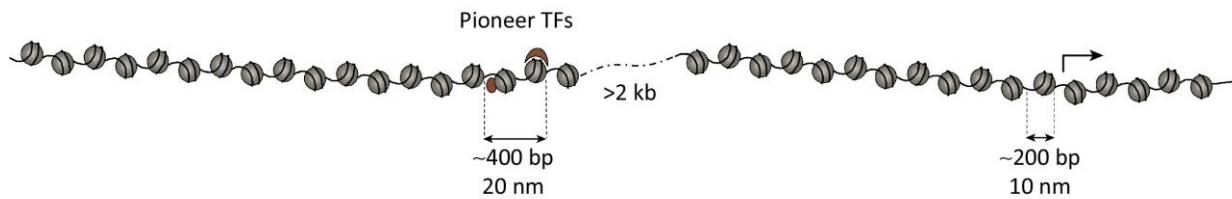


# Transcription factors lead to chromatin remodeling and RNA polymerase II recruitment

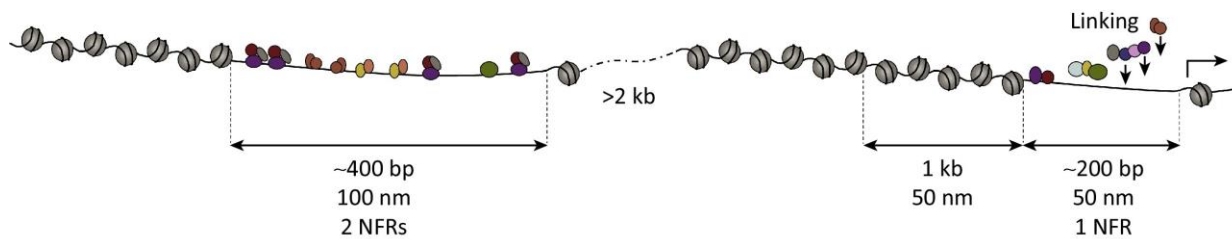
## Enhancer

## Promoter

(A) Enhancer priming



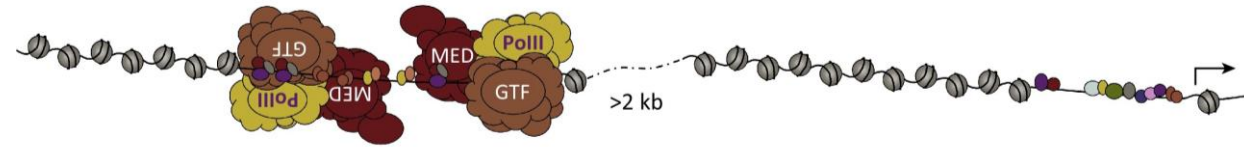
(B) Recruitment of large complexes and formation of open regions



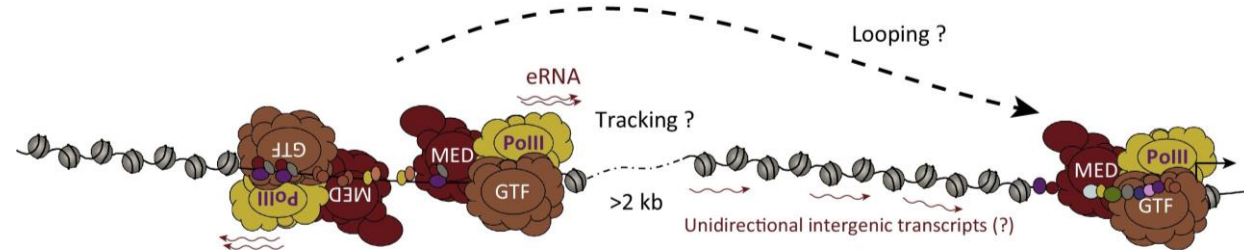
## Enhancer

## Promoter

(C) Recruitment of PIC and mediator at the enhancer



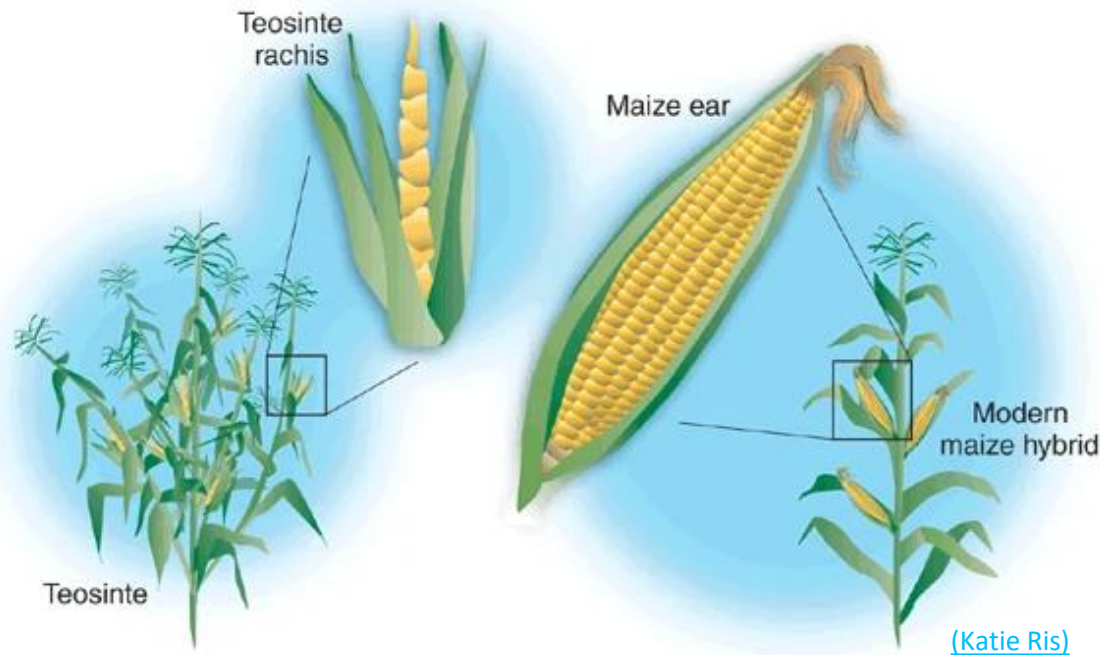
(D) Recruitment of PIC and mediator at the promoter



Trends in Genetics

[\(Vernimmen and Bickmore, 2015. Trends Genet.\)](#)

## There is genetic and molecular evidence for important enhancers in corn

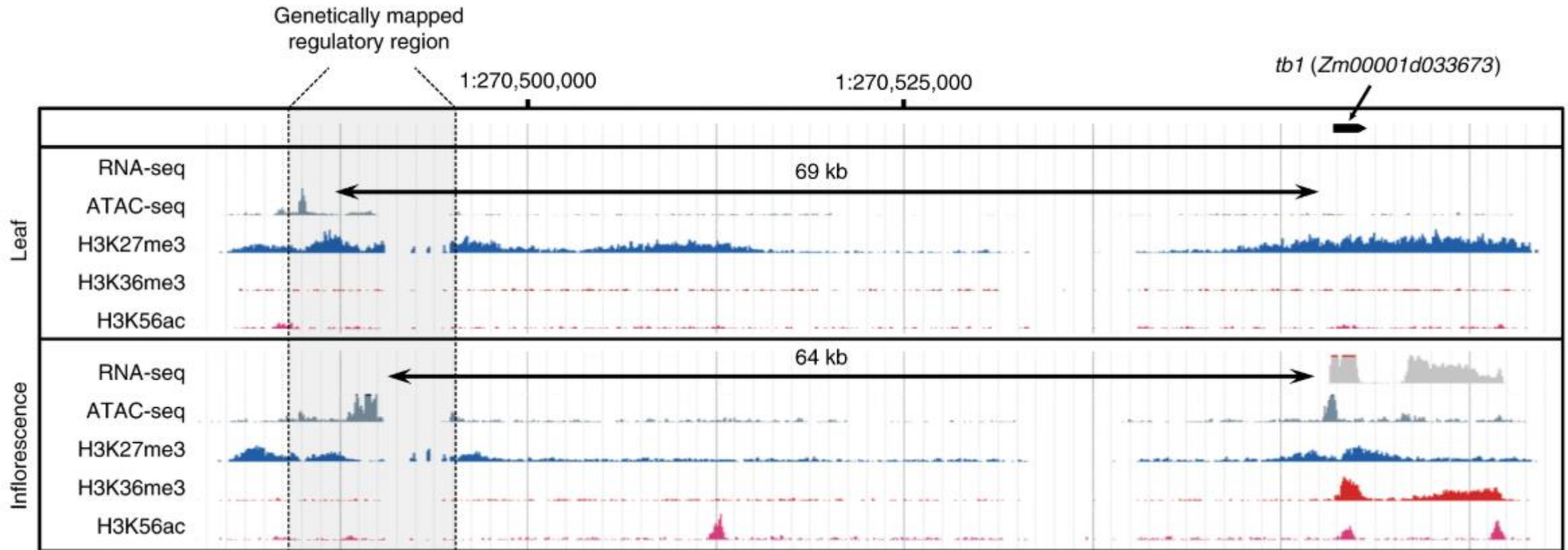


“Whereas levels of diversity in the coding portion are roughly comparable to those found in teosinte, diversity in the upstream noncoding region is significantly depleted... Thus, the coding region of *tb1* evolved neutrally during the domestication process of maize, whereas its upstream region was subjected to strong selection.”

[\(de Meaux, 2006. Nature Genetics.\)](#)

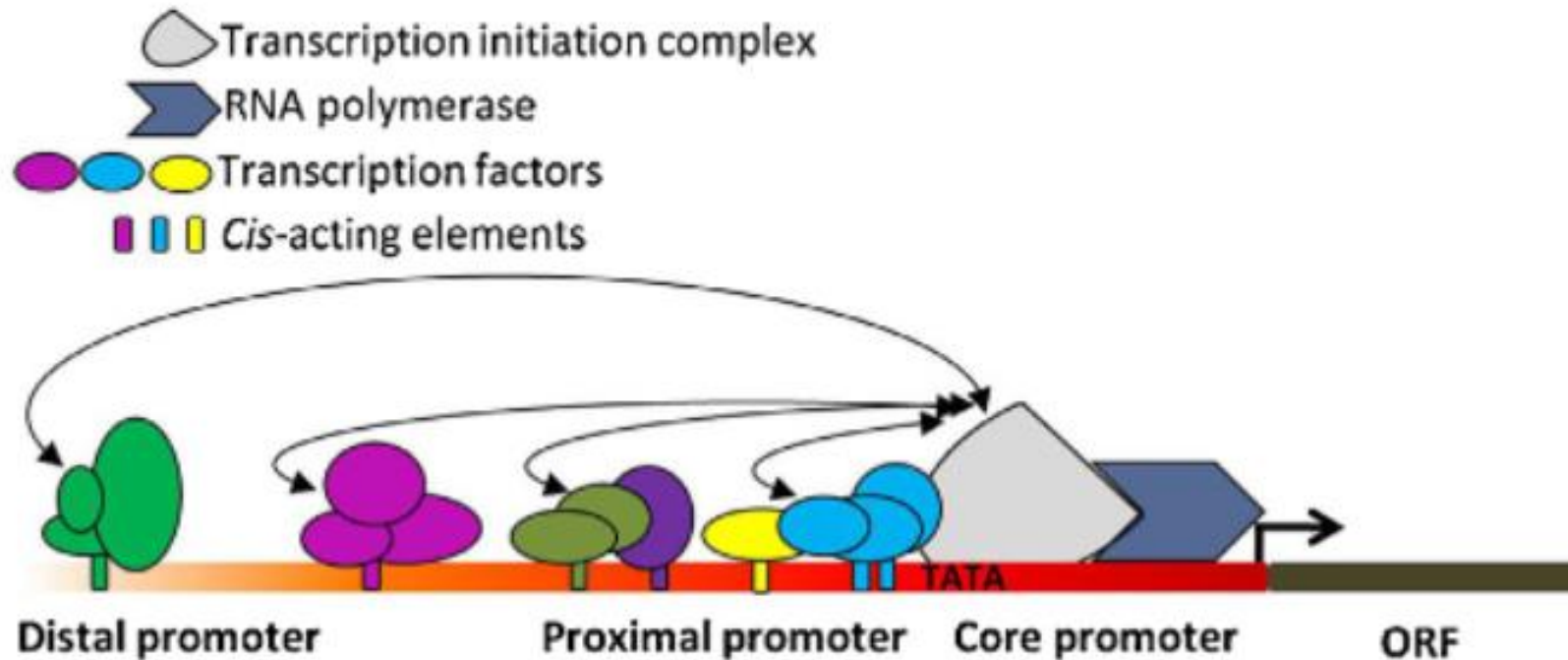
[\(Katie Ris\)](#)

# There is genetic and molecular evidence for important enhancers in corn



(Ricci et al, 2019. Nature Plants.)

Distal and core promoters are sequences upstream of genes bound by proteins to recruit RNA polymerase II



([Hernandez-Garcia and Finer, 2014. Plant Science](#))

# Promoters used in transgenic corn events submitted for deregulation in USA

Trade name	Developer	Promoters	Trade name	Developer	Promoters
Enogen™ Maize	Syngenta	Zm27kDa $\gamma$ -zein, CaMV 35S	InVigor™ Maize	Bayer Crop Science	P-CA55, CaMV 35S
32138 SPT maintainer	DuPont Pioneer	5126, ZmPg47, HvLTP2	InVigor™ Maize	Bayer Crop Science	pTA29, CaMV 35S, pTAC
Agrisure™ CB/LL	Syngenta	2X CaMV 35S	YieldGard™ VT Pro™	Monsanto	CaMV 35S, FMV 35S
Roundup Ready™ Maize, Agrisure™GT	Monsanto	OsActin	YieldGard™ VT™ Rootworm™ RR2	Monsanto	OsActin1, CaMV 35S
Agrisure™ RW	Syngenta	ZmMTL, ZmUbi1	Genuity® DroughtGard™	Monsanto	OsActin1, CaMV 35S
Agrisure® Duracade™	Syngenta	CMP, ZmUbi1	Roundup Ready™ Maize	Monsanto	CaMV 35S
Herculex™ I, Herculex™ CB	Dow & DuPont Pioneer	ZmUbi1, CaMV 35S		Monsanto	PCISV, AgUBQ
	Dow & DuPont Pioneer	CYMV, BSV, 2X ZmUbi1		Monsanto	CaMV 35S
	Dow & DuPont Pioneer	2X ZmUbi1, TaPOX, CaMV 35S	YieldGard™ Rootworm RW, MaxGard™	Monsanto	2X CaMV 35S
Agrisure™ Viptera	Syngenta	2X ZmUbi1, CaMV 35S	Roundup Ready™ Maize	Monsanto	2X CaMV 35S
	Genective S.A.	ScUbi4	YieldGard™, MaizeGard™	Monsanto	CaMV 35S
	Dow	ZmUbi1, CaMV 35S		Monsanto	3X CaMV 35S
Liberty Link™ Maize	Bayer Crop Science	CaMV 35S		Monsanto	3X CaMV 35S
Liberty Link™ maize	Bayer Crop Science	CaMV 35S		Monsanto	3X CaMV 35S
Roundup Ready™ 2 Maize	Monsanto	OsActin, CaMV 35S		Monsanto	3X CaMV 35S
	Syngenta	CMP, ZmUbi1, CaMV 35S			
	Syngenta	ZmUbi158, CaMV 35S			

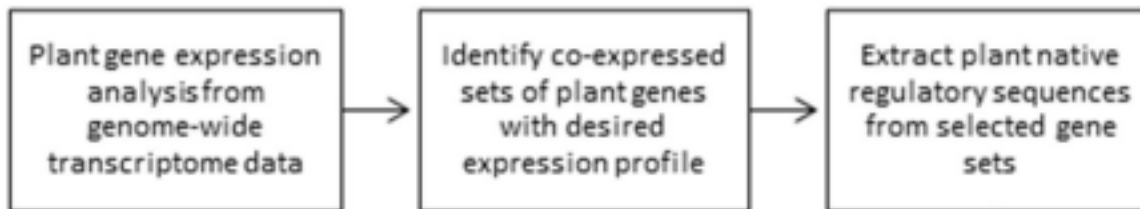
(Nuccio, 2018. Methods Mol Biol)



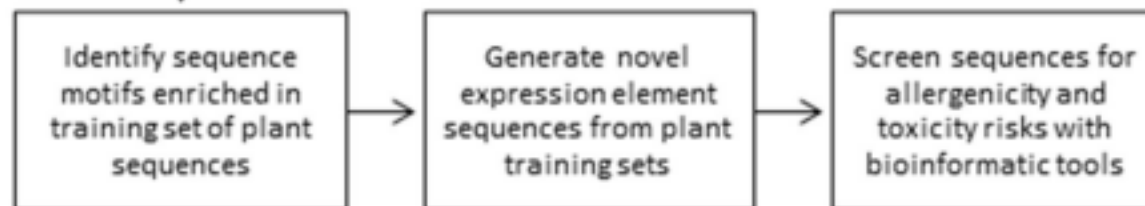


# Computational design of promoters and other expression elements

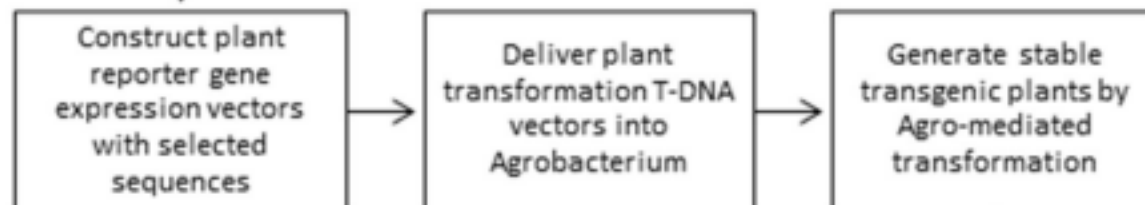
## Plant sequence training set identification



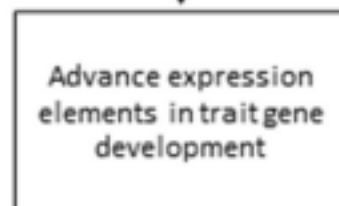
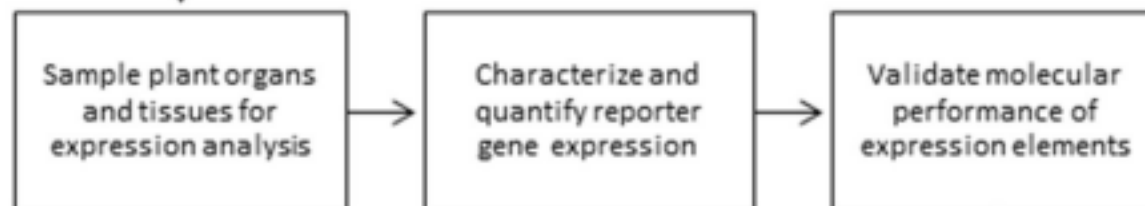
## Computational sequence generation



## Transgenic plant production

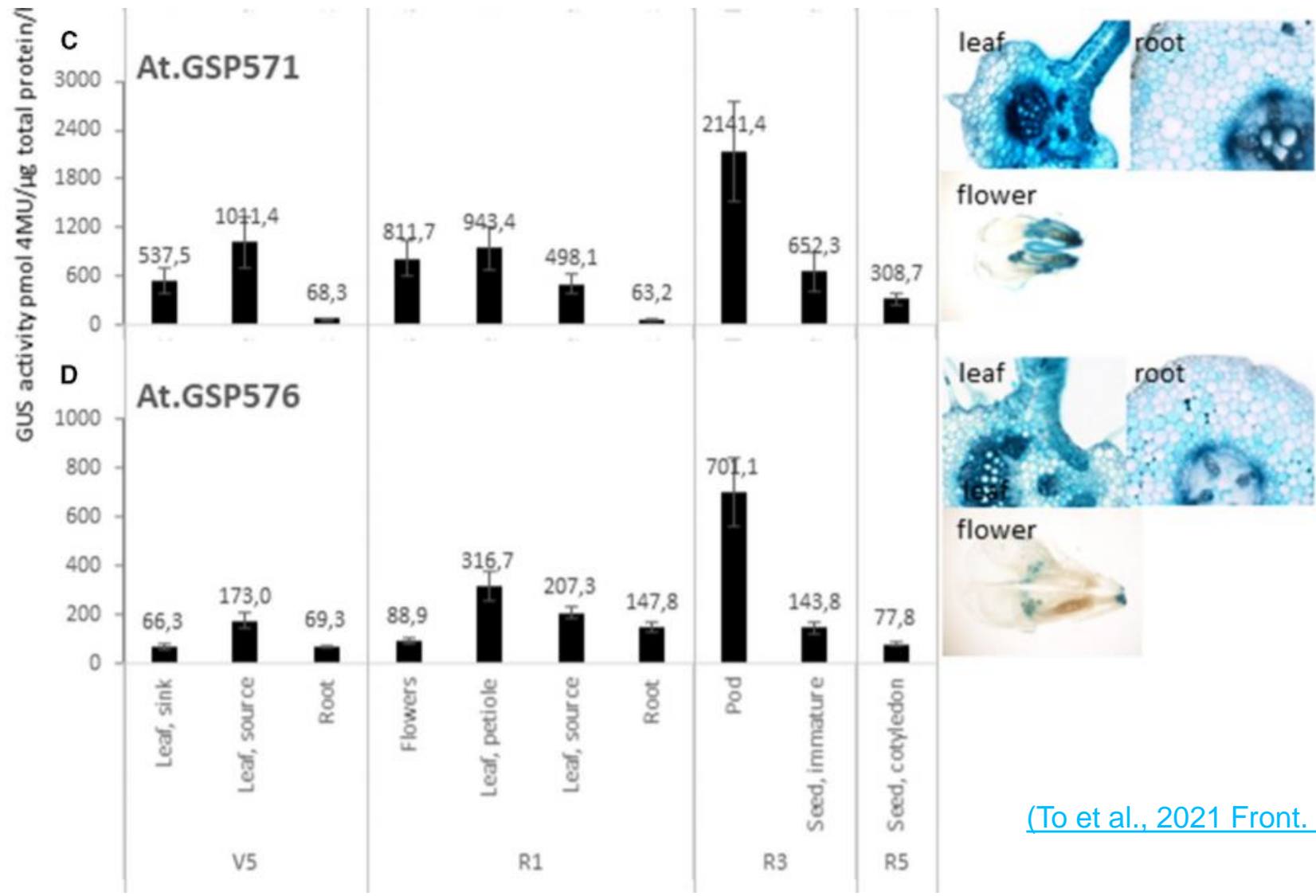


## Plant expression characterization



(To et al., 2021 Front. Plant Sci.)

# Computational design of promoters and other expression elements

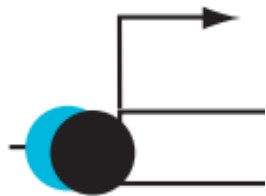


(To et al., 2021 Front. Plant Sci.)





## RNA Processing, including splicing, is co-transcriptional

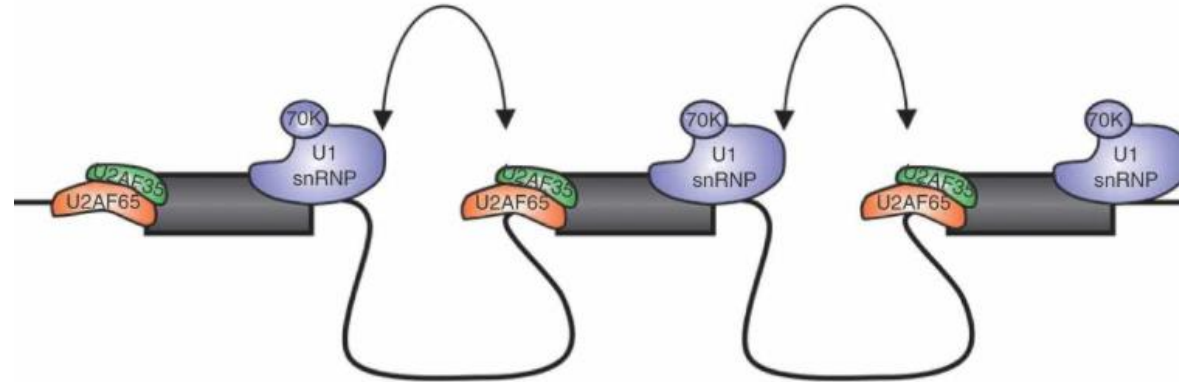


Pol II initiation .

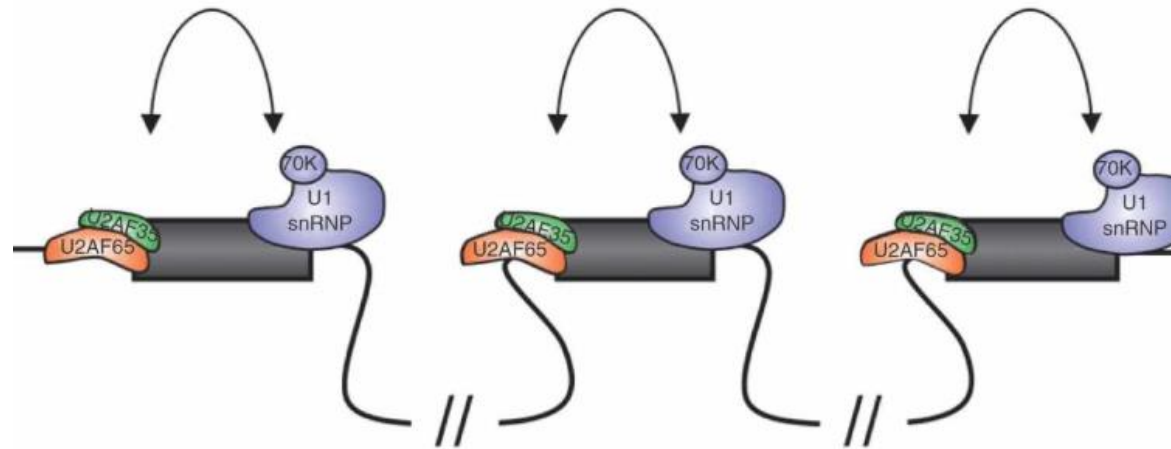
RNA . . . . .

[\(Neugebauer, 2002. J Cell Sci.\)](#)

# RNA Processing, including splicing, is co-transcriptional



Exon definition model

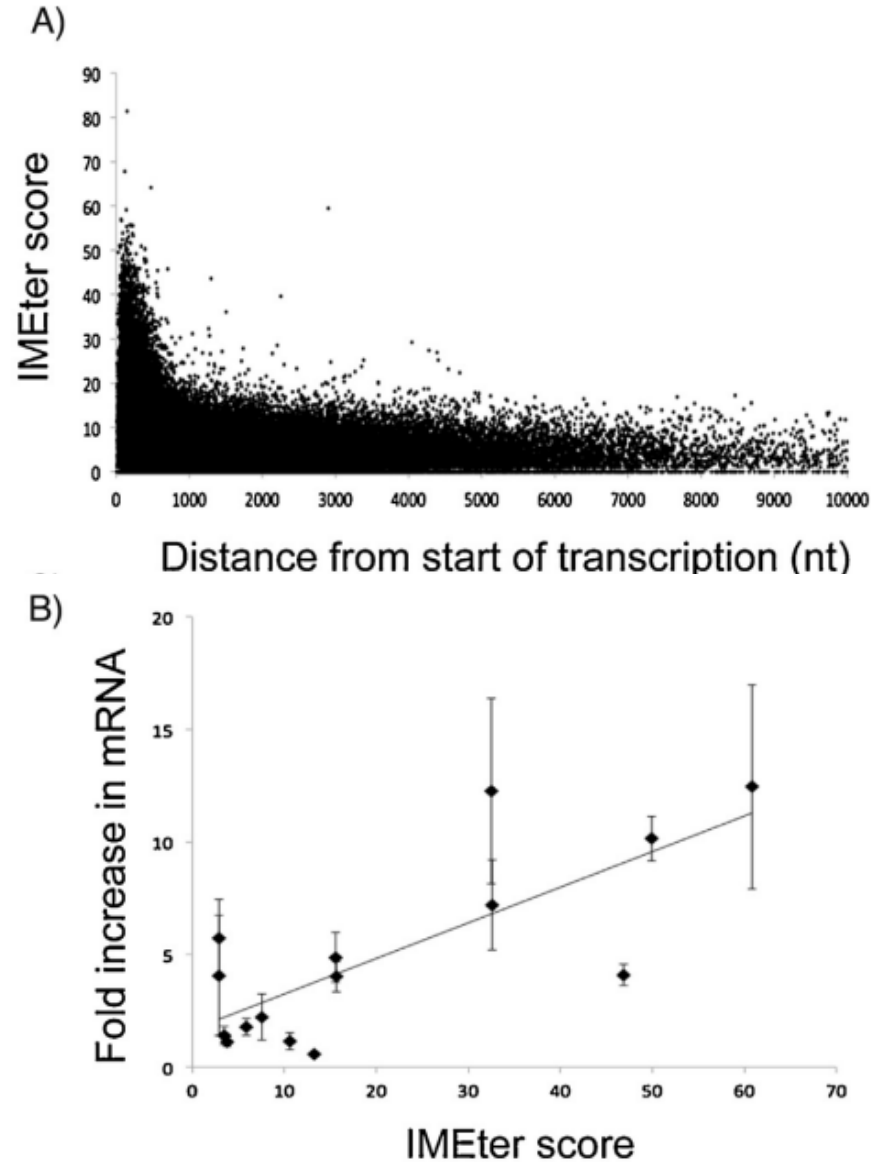


Introns > 250 bp

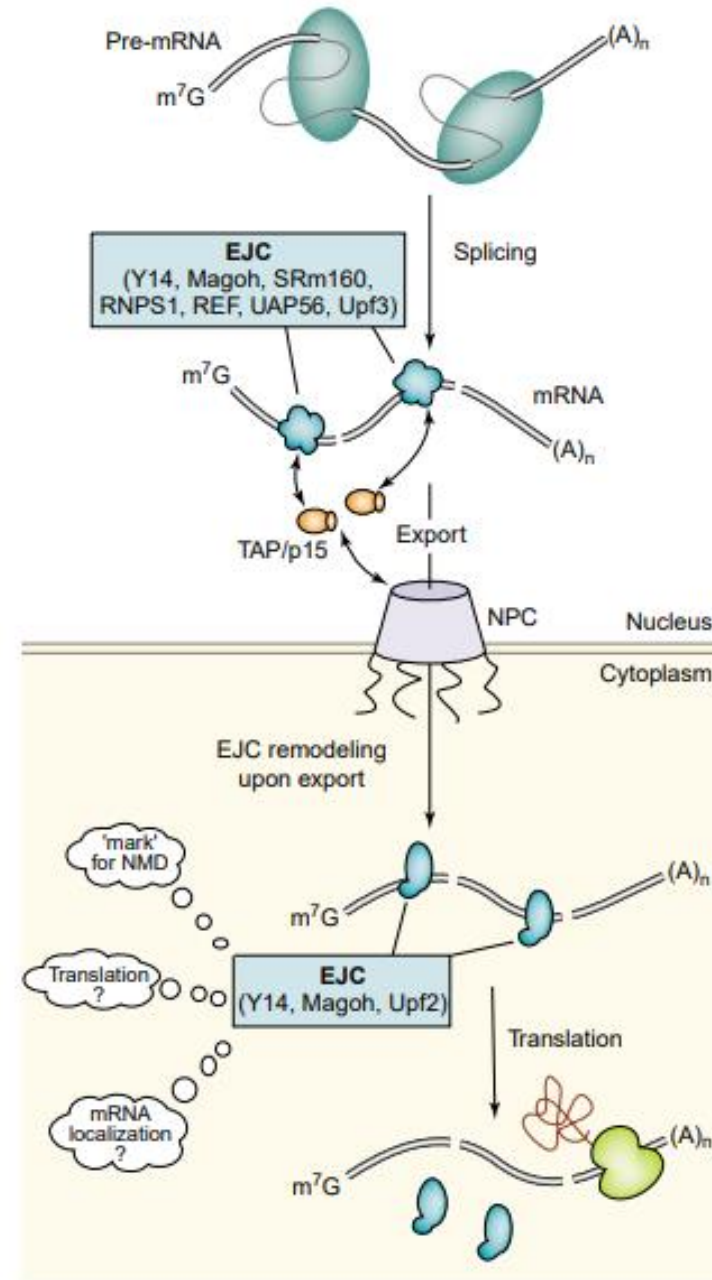
(De Conti, 2013. WIREs RNA.)



# Intron-mediated enhancement



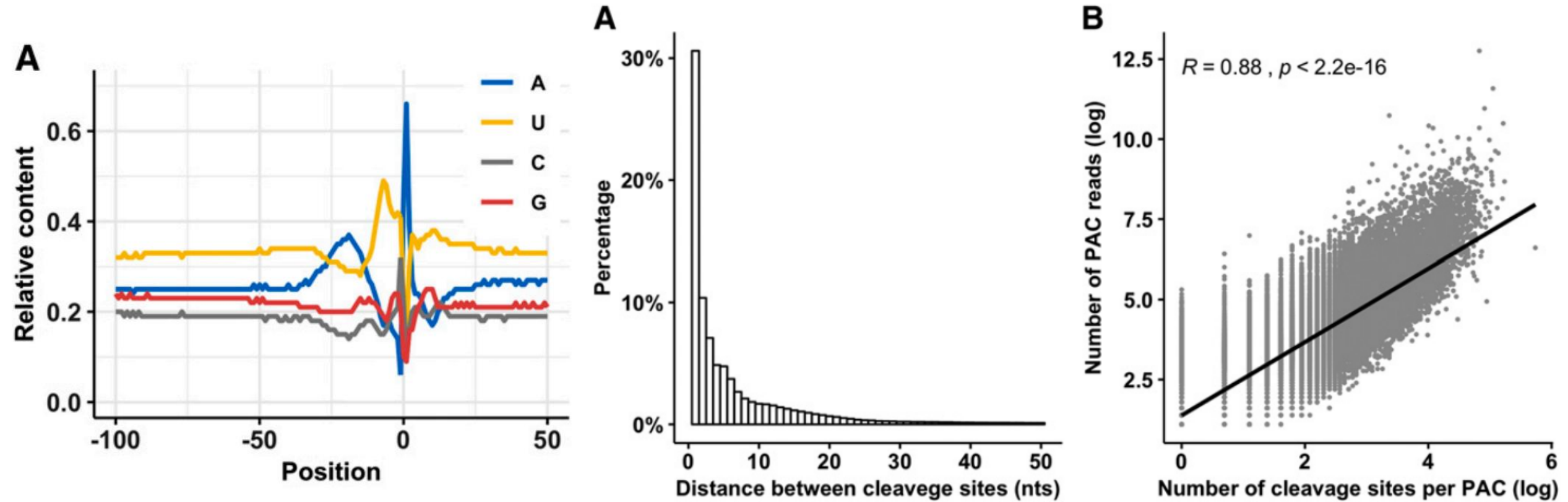
(Gallegos and Rose, 2015)



(Le Hir et al, 2003)



## Polyadenylation clusters in plants have core regulatory elements in 3' untranslated regions

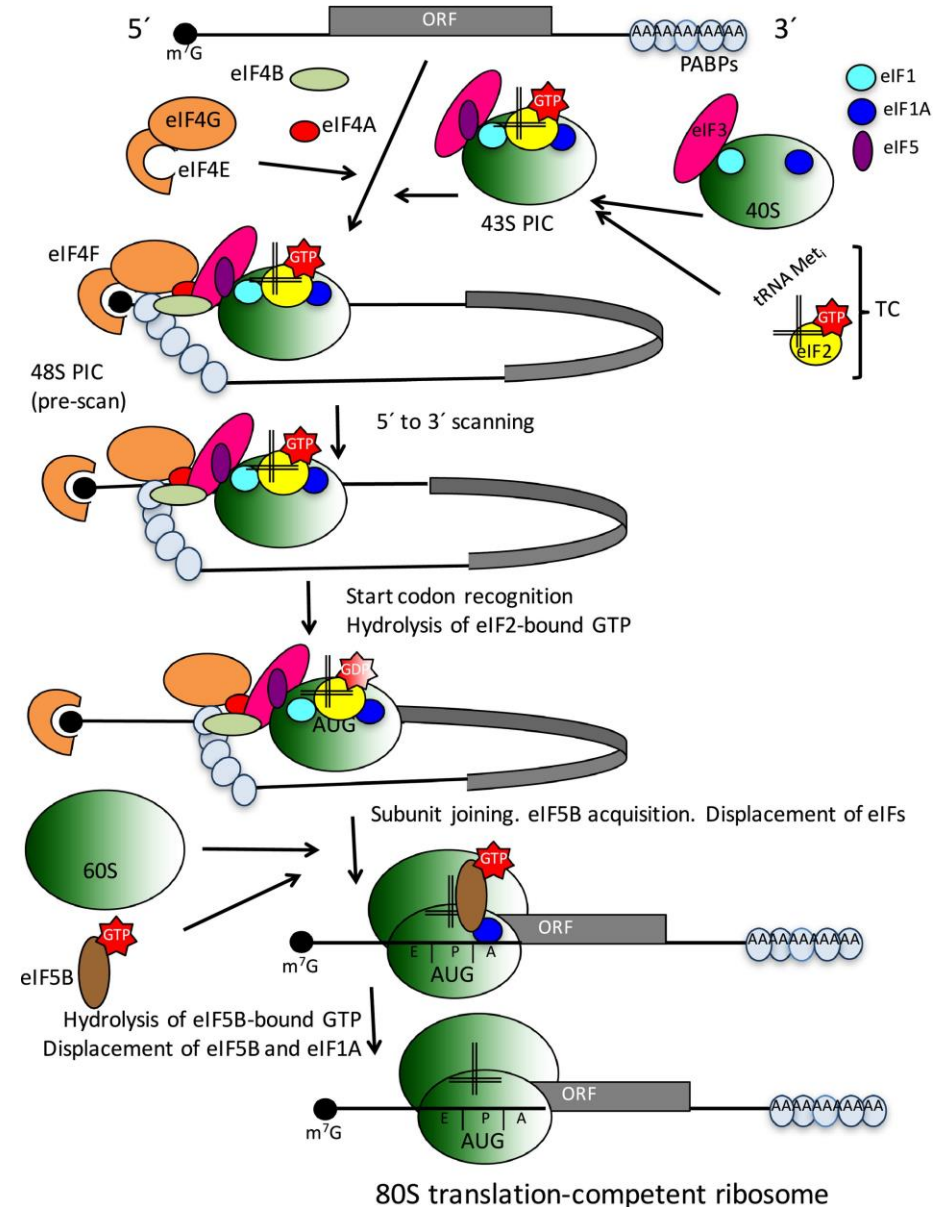


(Jafar et al, 2019)



# mRNA is translated by ribosomes

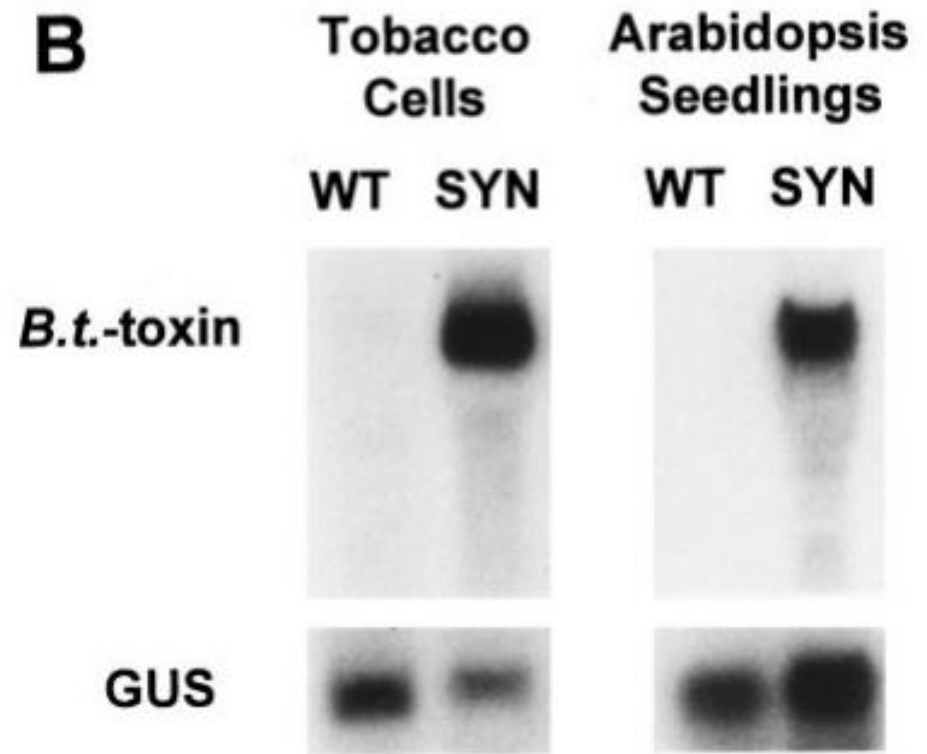
- The mRNA cap and polyA tail are both important for initial ribosome occupancy.
- Once loaded onto the 5' end of the mRNA, the 40S ribosome scans from the 5' end for the first ATG start codong.
  - This is why upstream ATGs (uORFs) downregulate expression, and also why upstream ATGs can be a risk factor for unexpected peptides.
- During translocation, the mRNA moves through the ribosome in codon triplets.



# Adapting bacterial genes to plant codon usage increases gene expression

**Table 1.** Summary of differences between the wild-type and synthetic *B.t.-toxin* genes

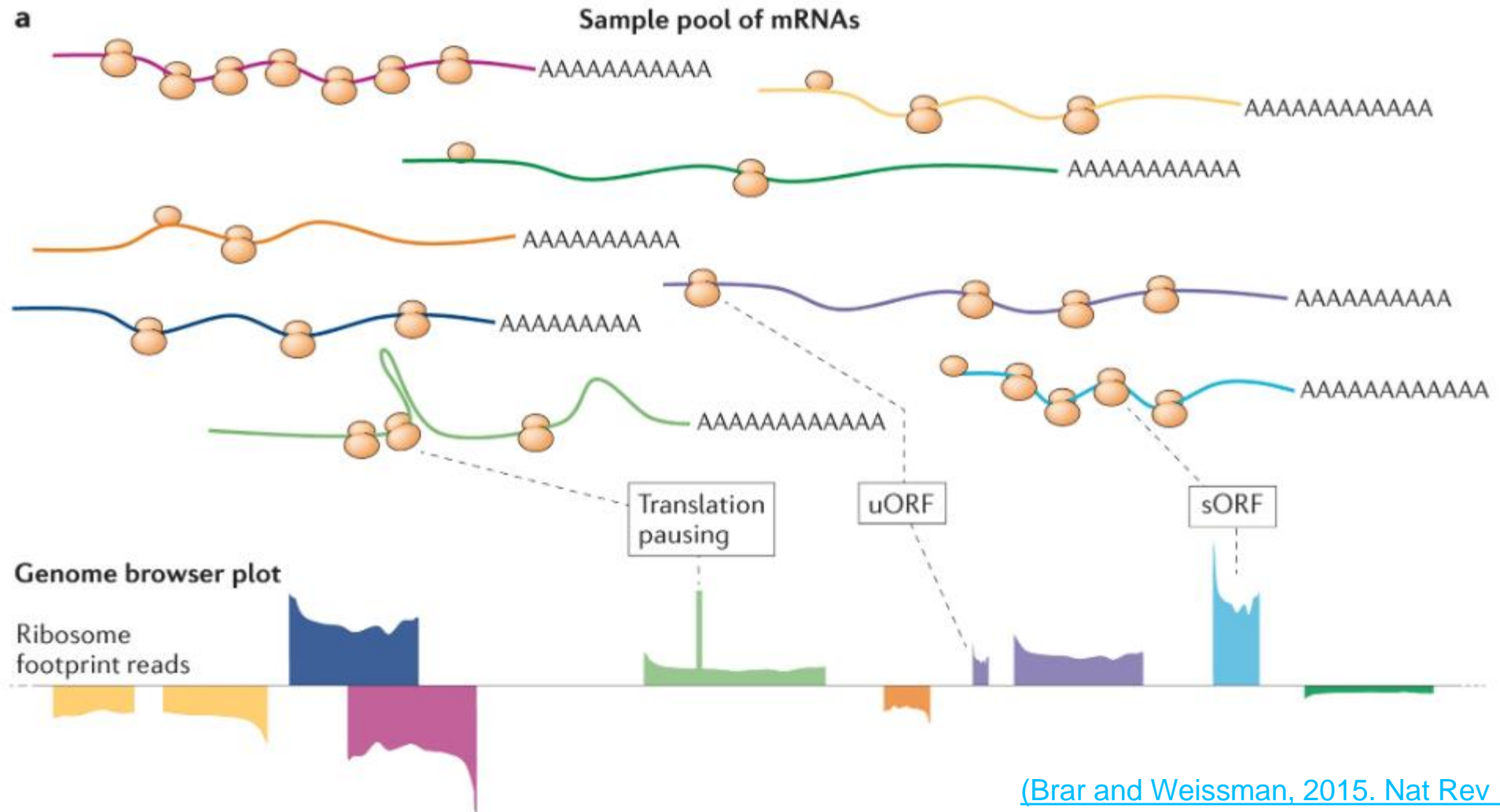
Segment	Length	Nucleotides Changed	Codons Changed	Initial G/C Content	Final G/C Content
	<i>bp</i>	<i>no.</i>		<i>%</i>	
1	274	82	75/91	38	66
2	375	106	96/125	38	65
3	531	167	146/177	37	64
4	653	190	179/217	38	63
Total	1833	548	496/610	38	64



[\(De Rocher, et al., 1998\)](#)

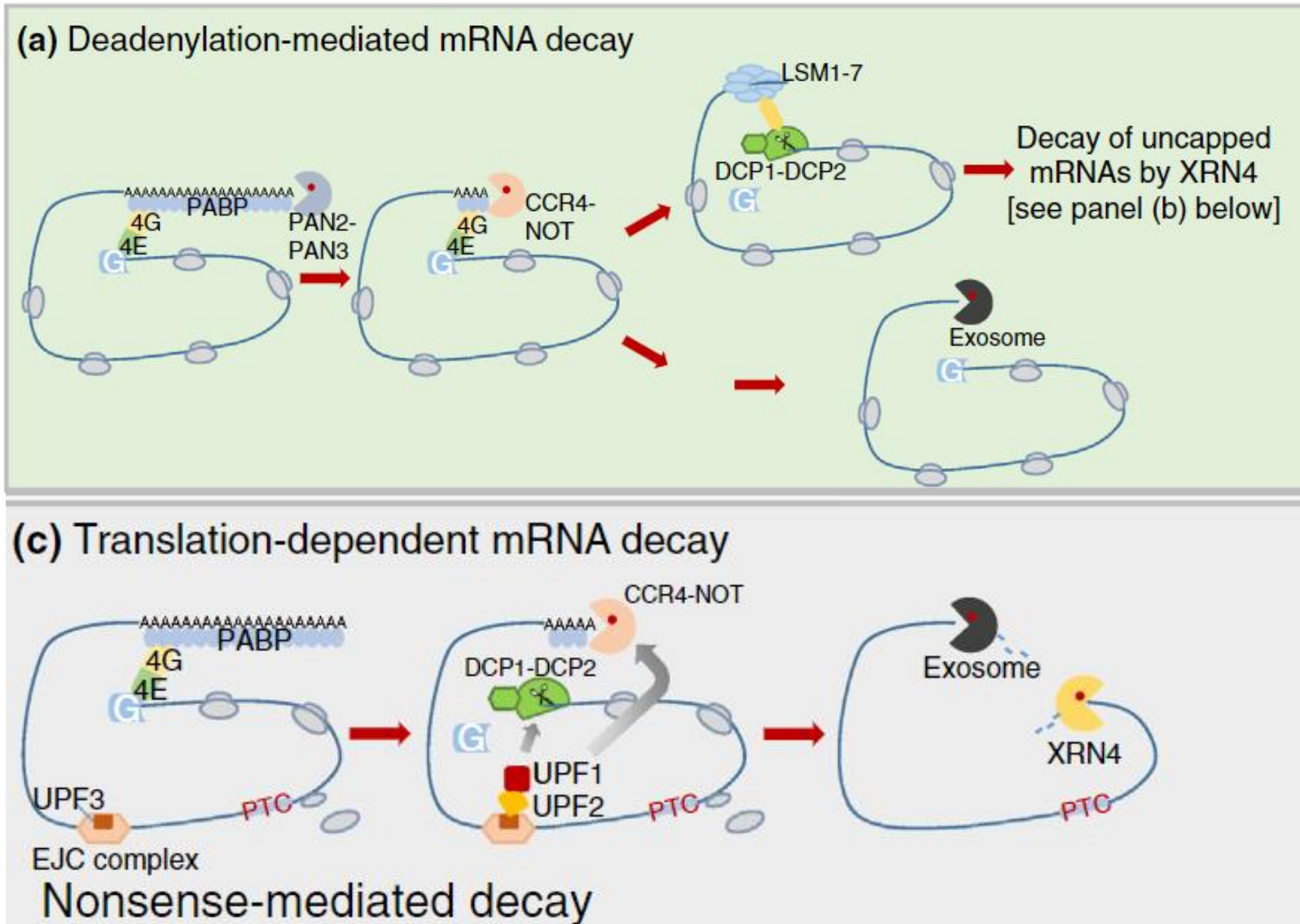


# Ribo-seq lets us visualize ribosome occupancy on mRNA



(Brar and Weissman, 2015. Nat Rev Mol Cell Biol.)

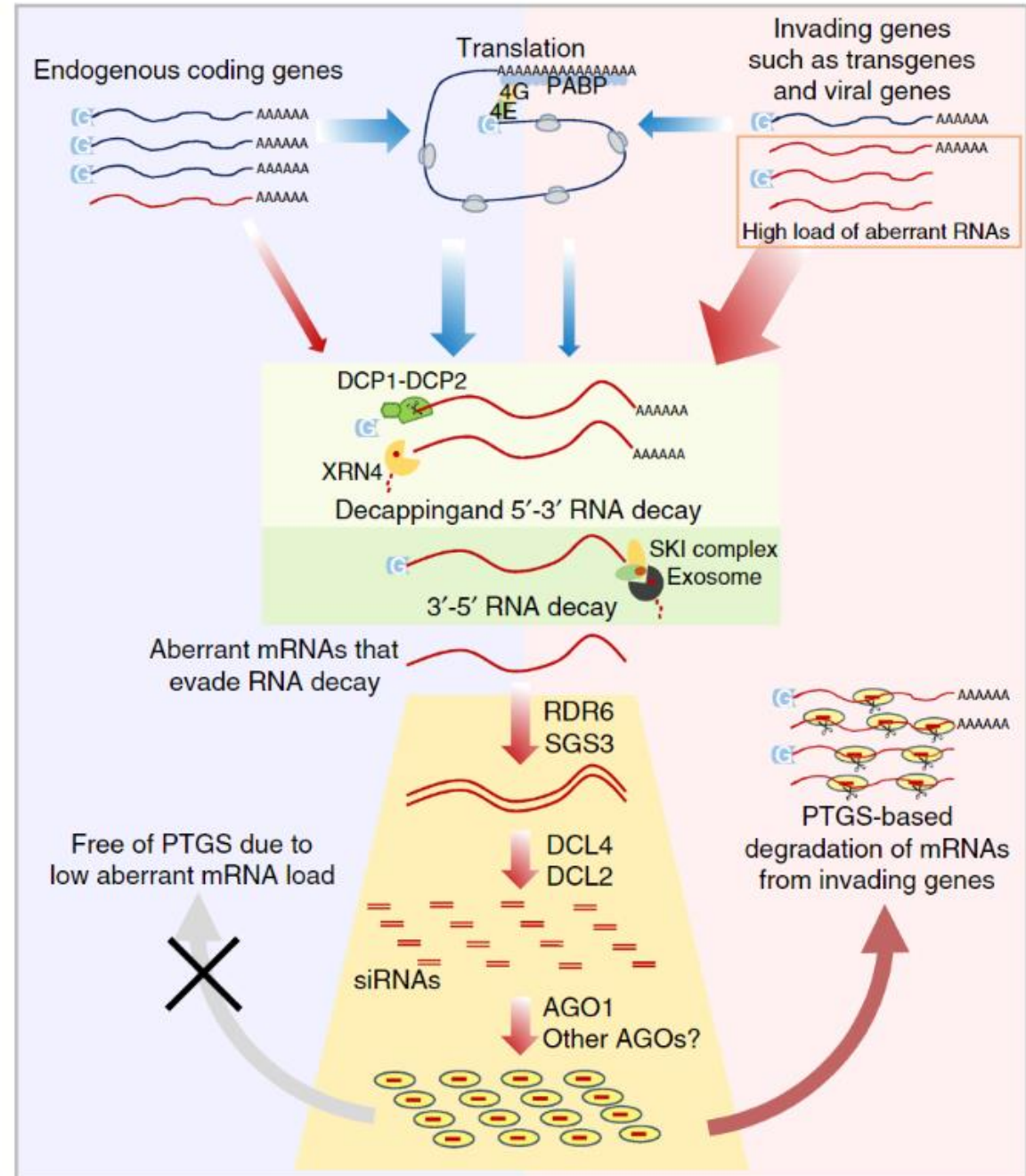
# RNA degradation pathways



(Zhang and Guo, 2017. Curr Opin Plant Biol.)

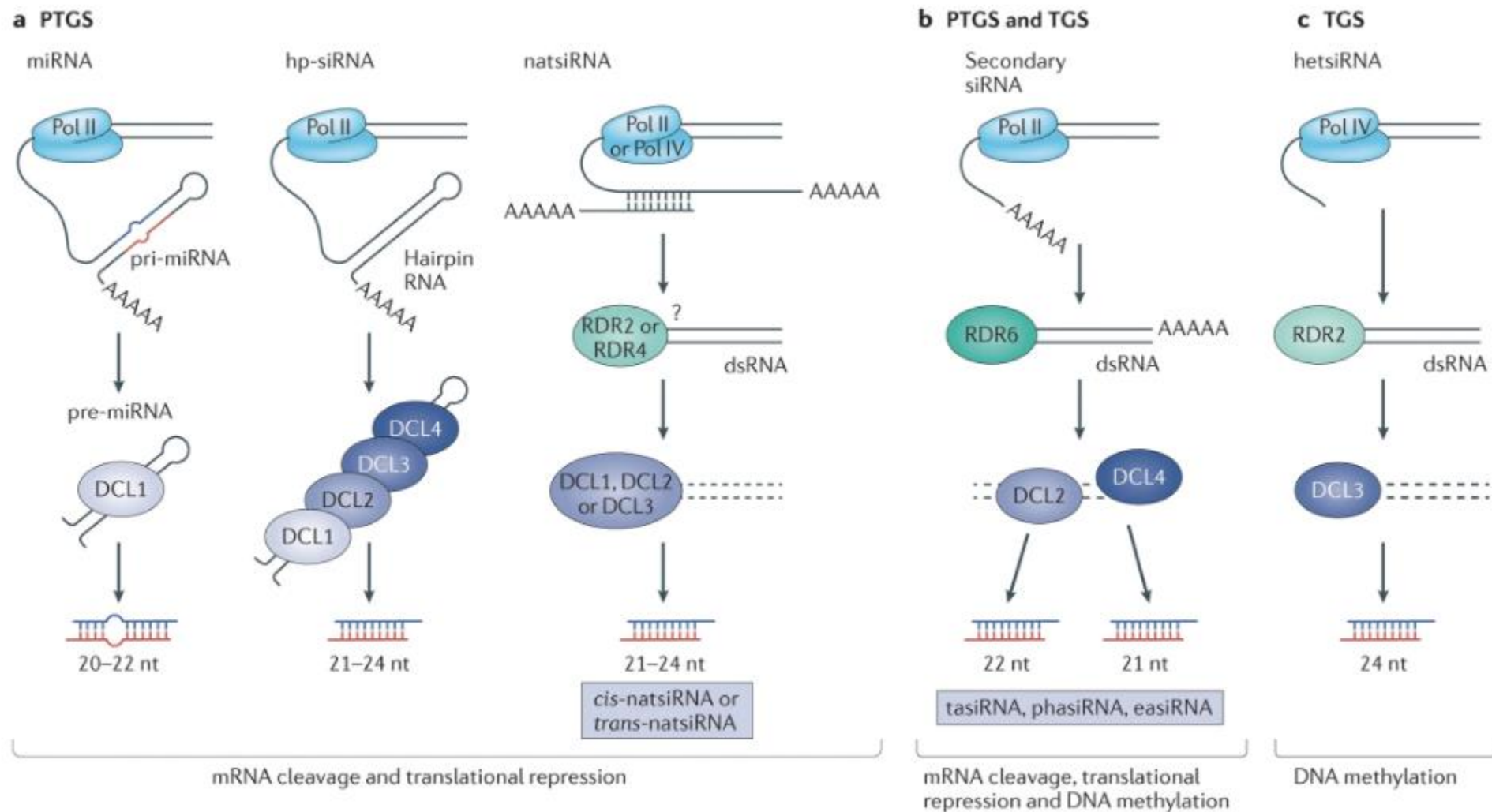
# RNA degradation

- Balanced mRNA decay prevents post-transcriptional gene silencing.
- If the decay mechanisms are not in balance with transcript generation, RNA-dependent RNA polymerases can kick off a silencing pathway.
- Design and testing of 3' untranslated regions, aka “terminators,” is important for predictable gene expression. Motifs in these designs may increase or decrease RNA stability.



(Zhang and Guo, 2017. Curr Opin Plant Biol.)

# Small RNA pathways in plants

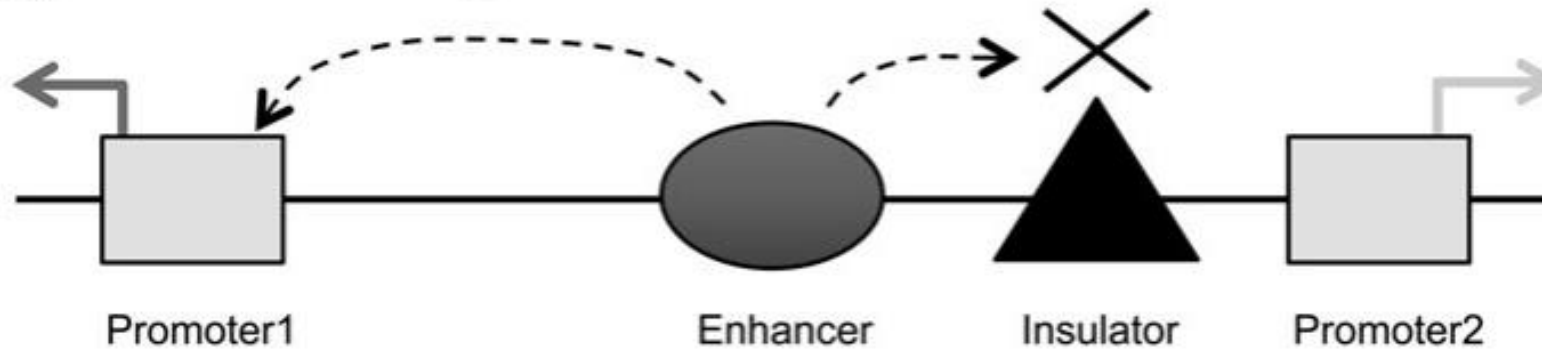


(Borges and Martienssen, 2015. Nat Rev Mol Cell Biol.)

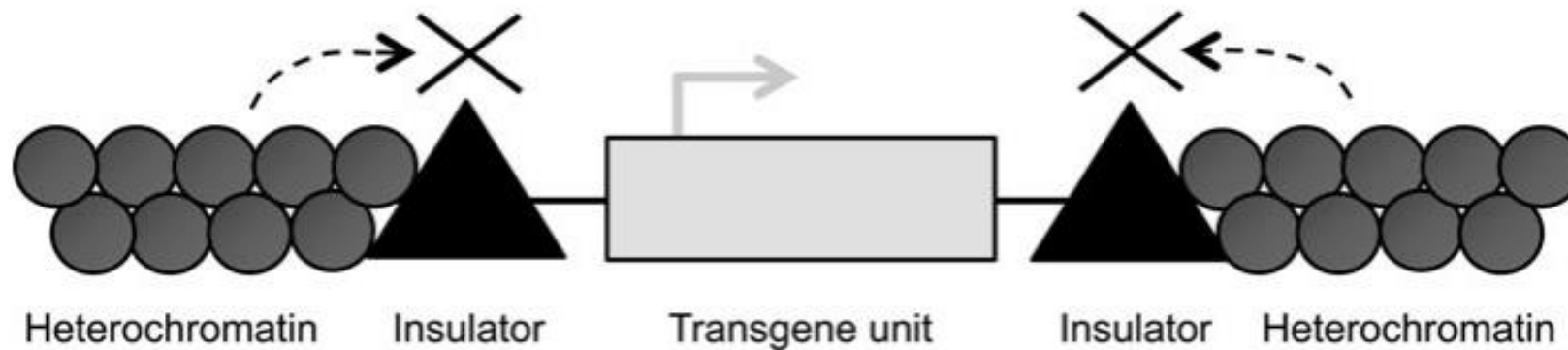


## Transgenes that are stacked on the same vector may affect each other's expression

**(a) Enhancer-blocking insulation**



**(b) Barrier insulation**



[\(Singer et al., 2012 Plant Cell Rep.\)](#)





# Gene expression: overall themes

- // Genes are expressed via structured complexes of nucleic acids and proteins
- // Gene expression processes are coupled and highly regulated
- // We can use expression regulatory elements, such as promoters, to recapitulate some gene expression patterns with transgenes in plants



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# *Thank you!*



Any questions?

