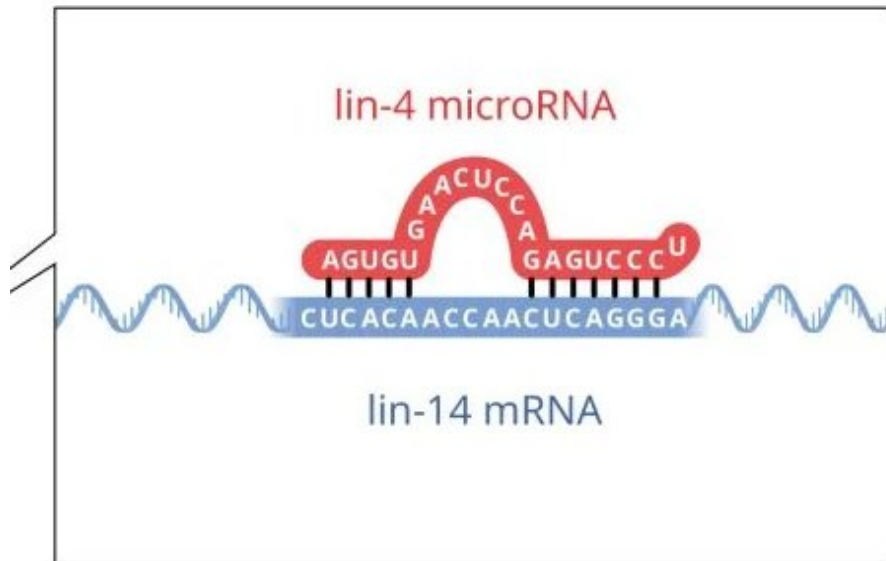


Mécanismes de dégradation de l'ARN chez les eucaryotes : histoires de coiffe et de queue poly(A)

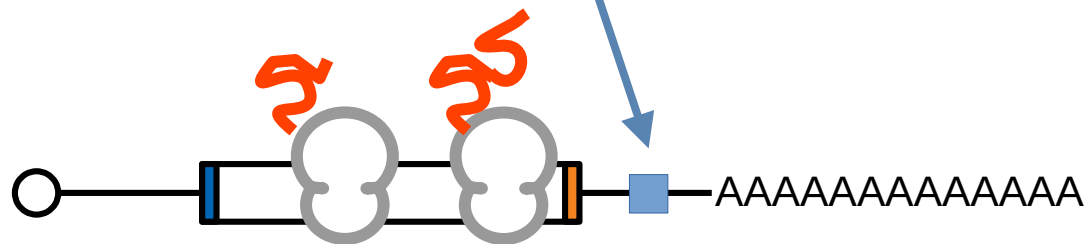


<https://www.nobelprize.org>

The Nobel prize in Physiology or
Medicine 2024

For the discovery of microRNA
and its role in post-transcriptional
gene regulation

Victor Ambros and Gary Ruvkun



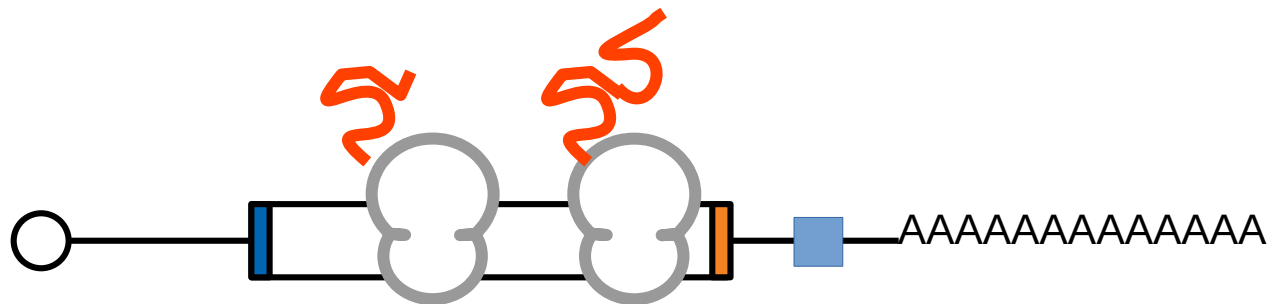
News and views:

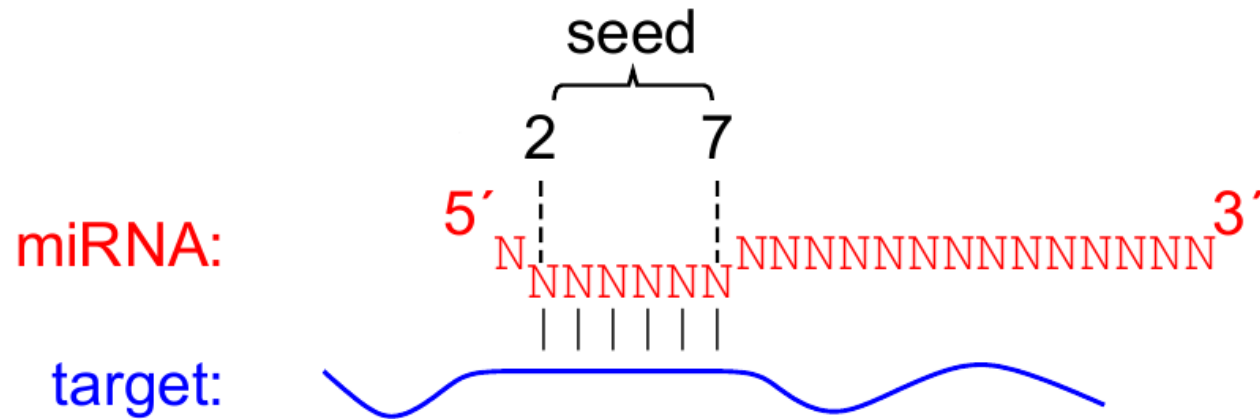
How comparative genomics can identify **functional mRNA targets** of miRNA ?

How **low affinity** of a protein to RNA can be useful to distinguish intronless from intron-containing genes ?

Main topic:

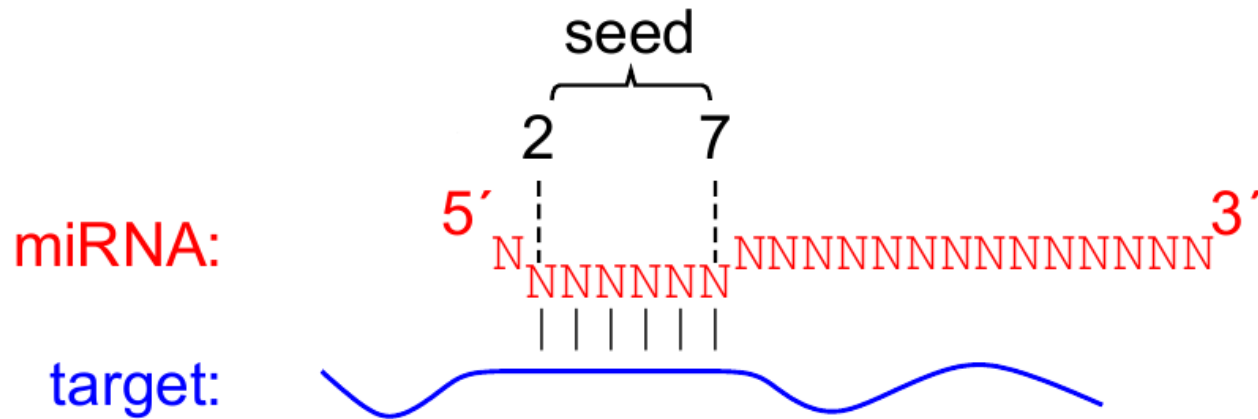
What role the poly(A) tail and cap play in mRNA degradation in yeast ?





Conservation during evolution

List of 3'UTRs for "predicted mRNA targets"



Most mammalian mRNAs are conserved targets of microRNAs

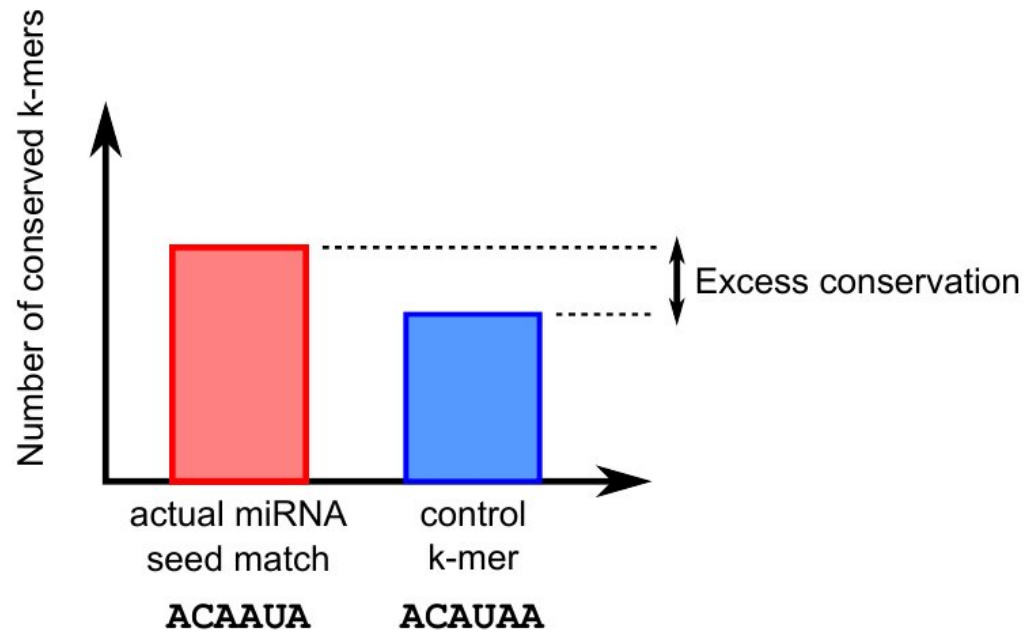
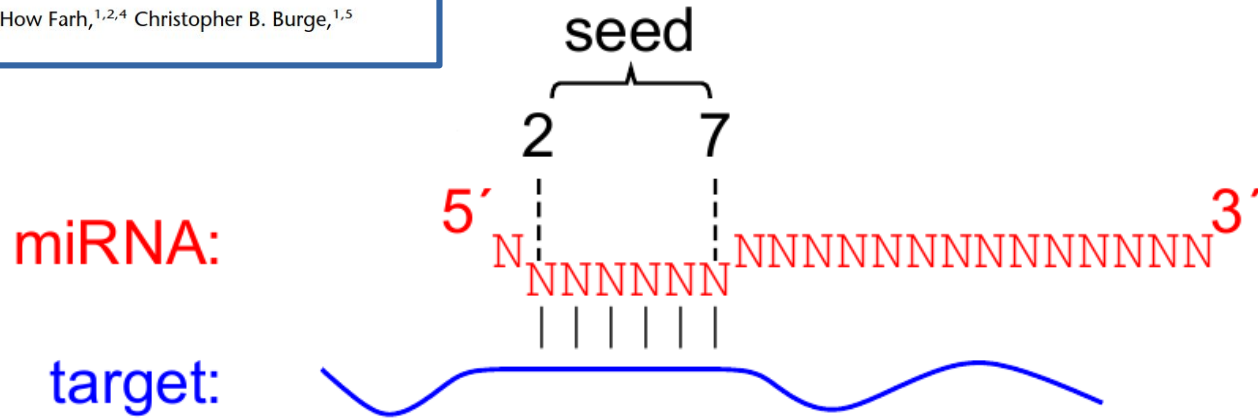
Robin C. Friedman,^{1,2,3} Kyle Kai-How Farh,^{1,2,4} Christopher B. Burge,^{1,5}
and David P. Bartel^{1,2,5}

2009

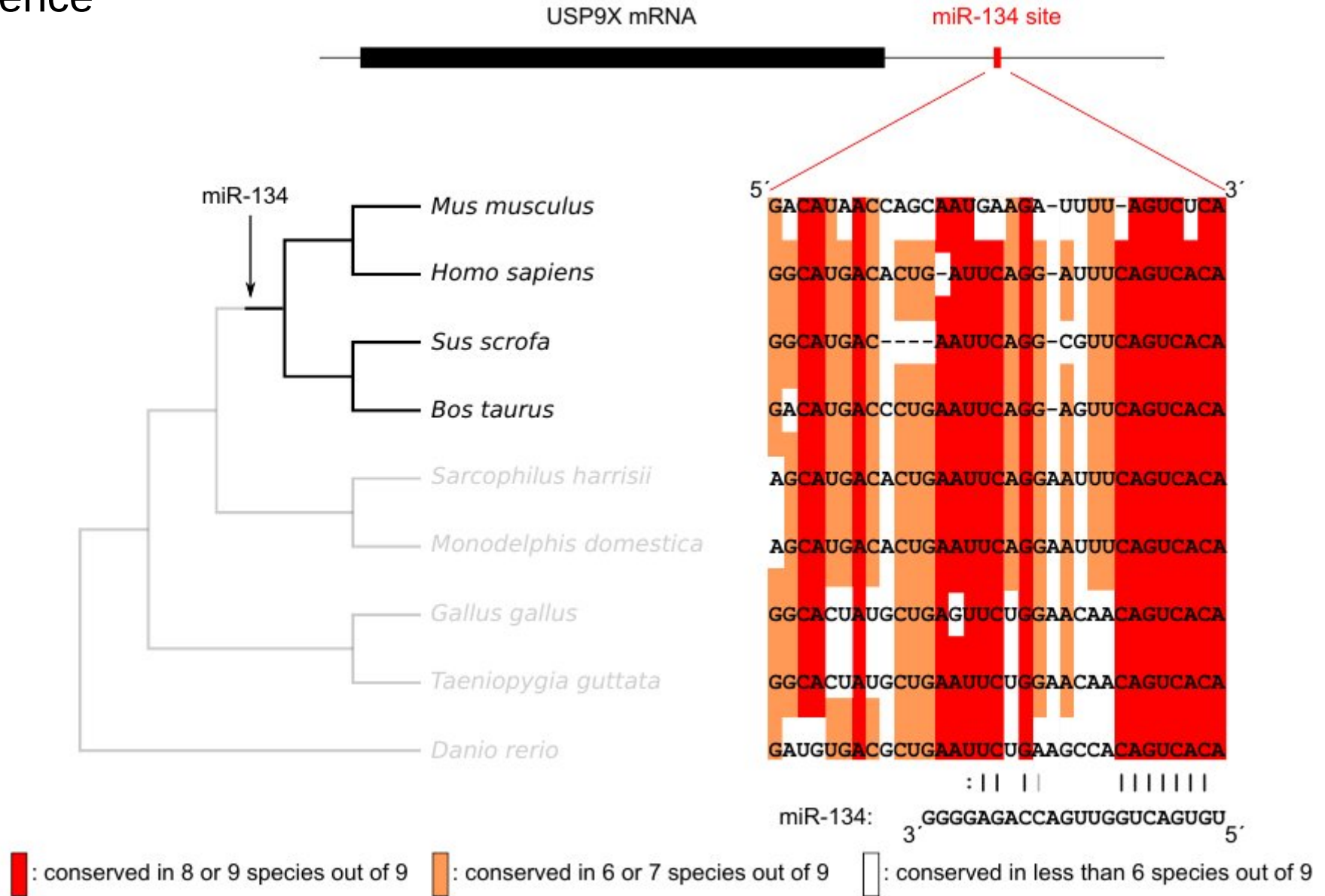
List of 3'UTRs for "predicted mRNA targets"

Most mammalian mRNAs are conserved targets of microRNAs

Robin C. Friedman,^{1,2,3} Kyle Kai-How Farh,^{1,2,4} Christopher B. Burge,^{1,5} and David P. Bartel^{1,2,5}

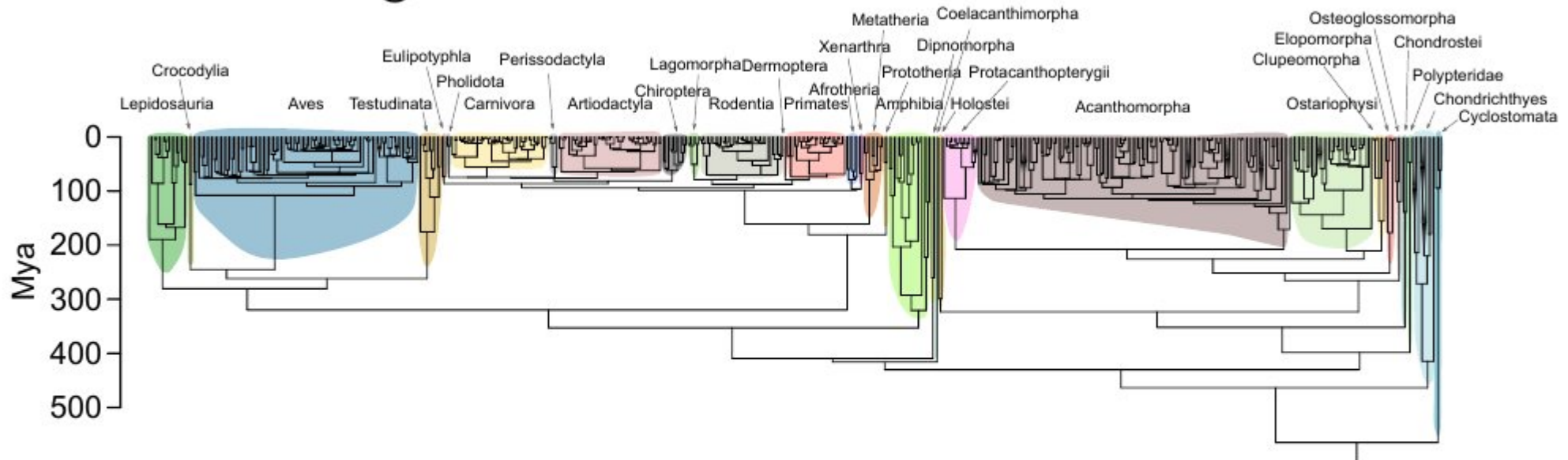


Conservation of “seed” regions can be independent of miRNA presence



Strategy to identify functional conservation of miRNA sites

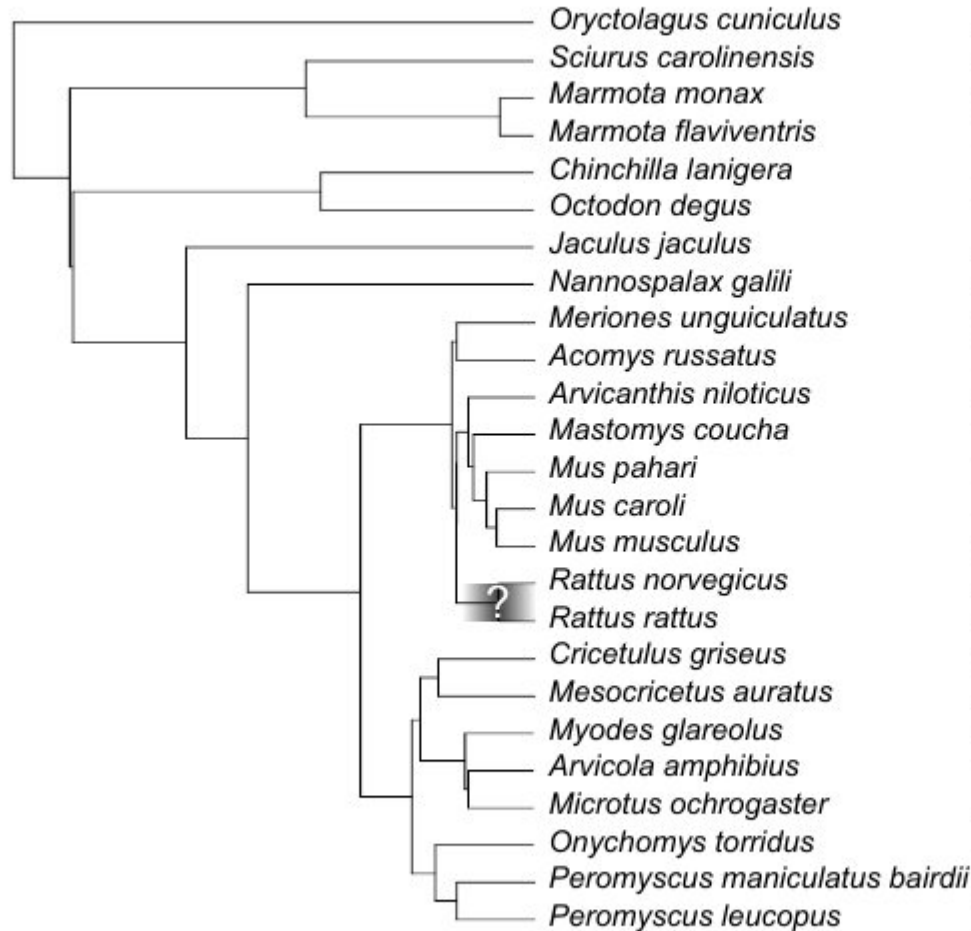
522 vertebrate genomes:



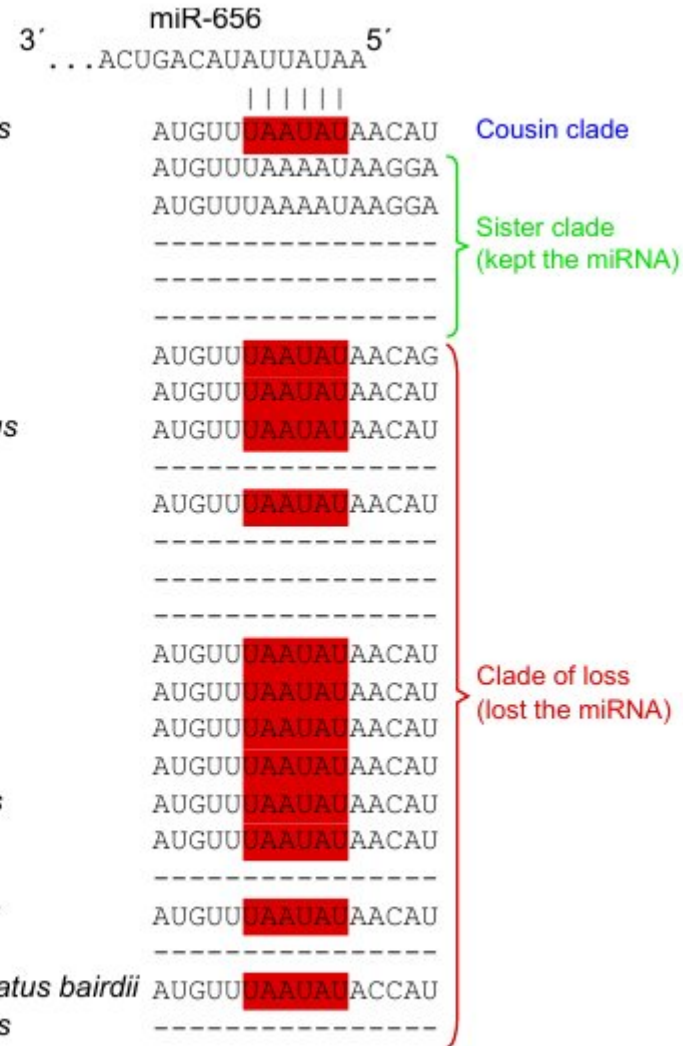
848 best annotated vertebrate miRNA families.

What happened to the predicted miR-656 target sequence after miR loss ?

SOX4 ancestral site #2:



10⁷ years



Conservation even without the miR...

Conclusions

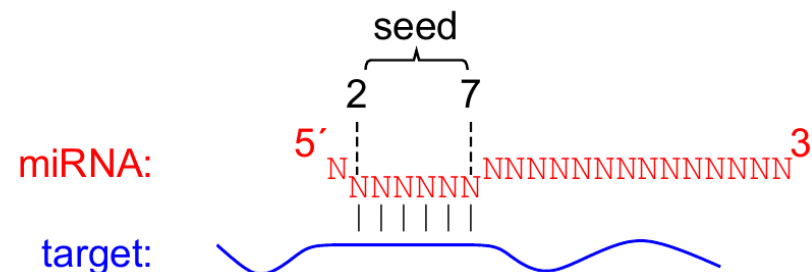
High-throughput experimental target identification captures many false positives...

... but comparative genomics too!

Improvement: identification of miRNA-independent conservation of binding sites.

On the analyzed examples: **most conserved seed matches are not conserved because of the miRNA!**

→ A large part of the miRNA literature probably needs a cleanup.



News and views:

How comparative genomics can identify **functional mRNA targets** of miRNA ?

How **low affinity** of a protein to RNA can be useful to distinguish intronless from intron-containing genes ?

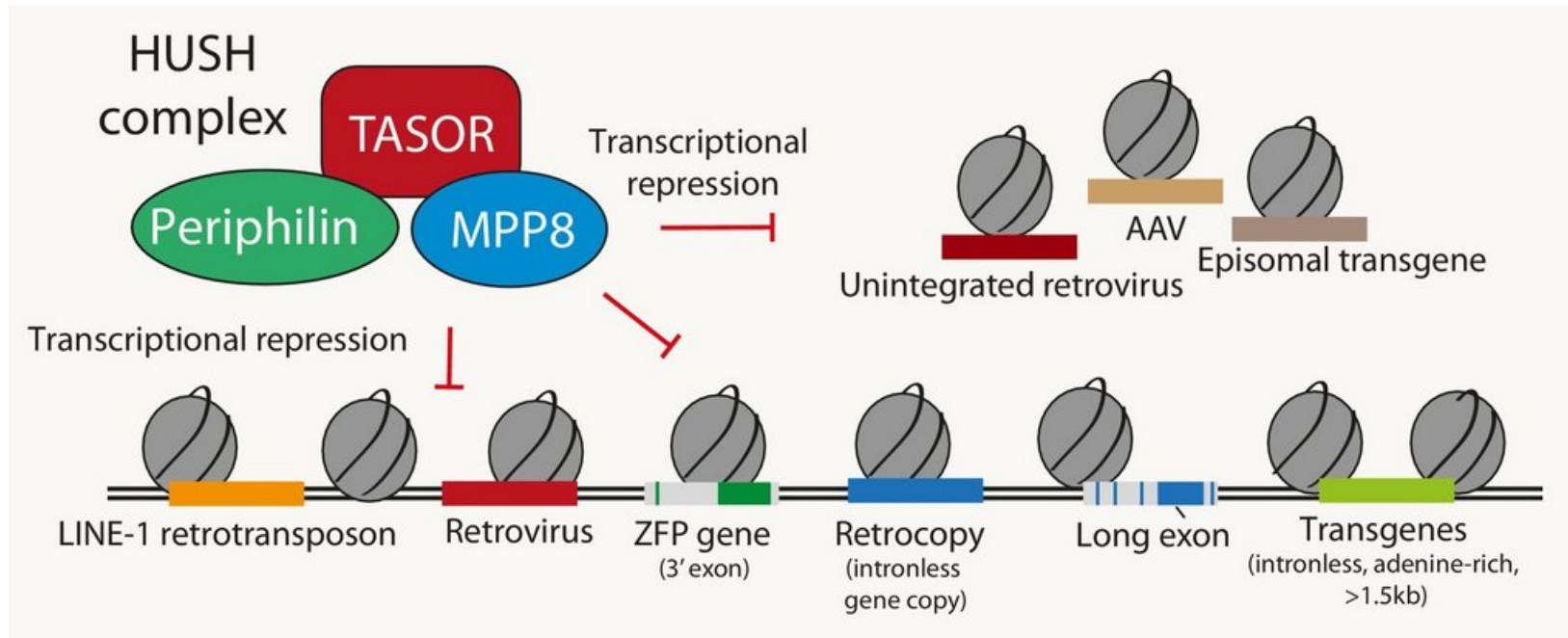
42% of the human genome is **retroelement***-derived

Intronless – but not highly expressed! Why ?

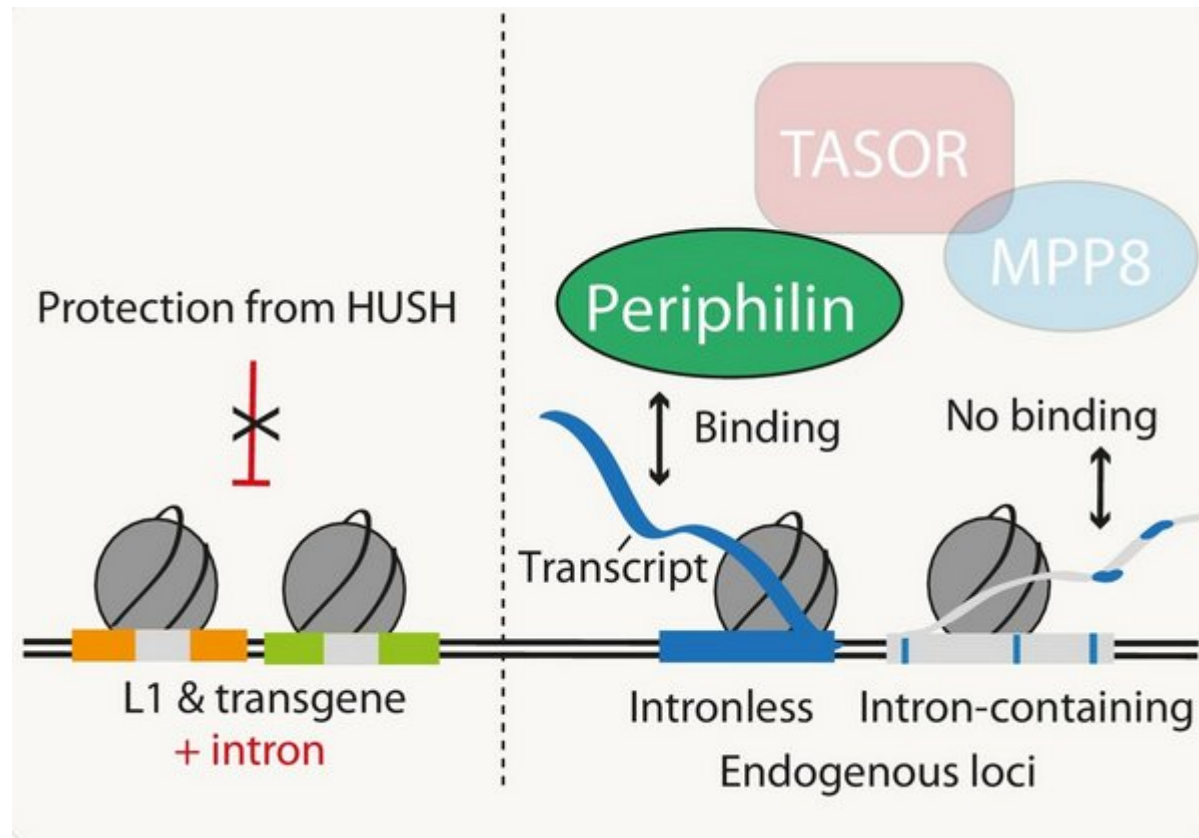
* mobile DNAs which replicate through an RNA intermediate

Marta Seczynska and Paul J.
Lehner *Trends in Genetics* 2023

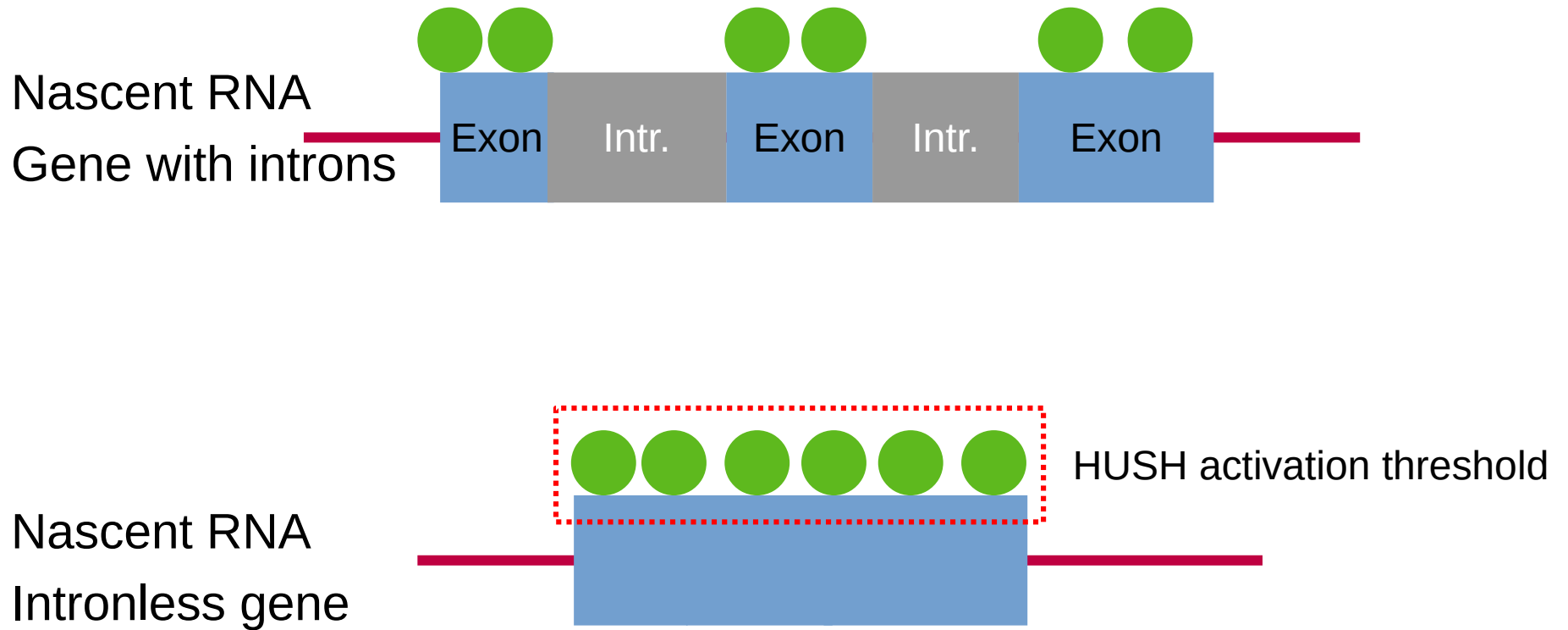
Human silencing hub (HUSH) – avoid transcription by binding to nascent RNA (?)



Periphilin binds to nascent RNA from genes without introns



Periphilin binds to nascent RNA from genes without introns, but independent of splicing!



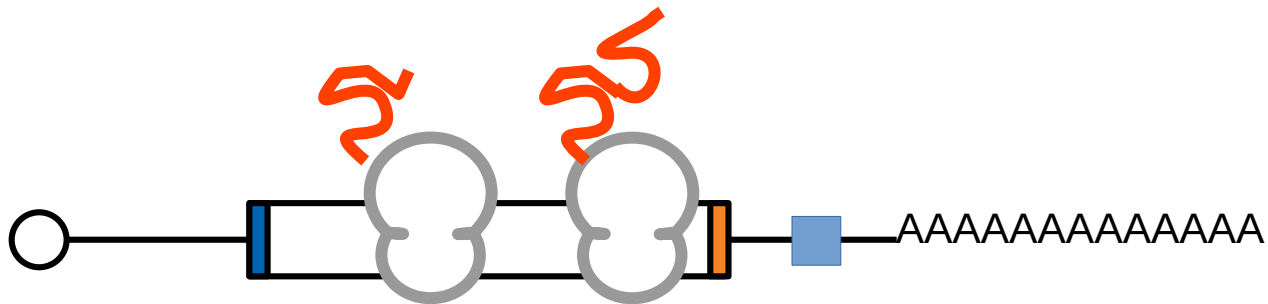
News and views:

How comparative genomics can identify **functional mRNA targets** of miRNA ?

How **low affinity** of a protein to RNA can be useful to distinguish intronless from intron-containing genes ?

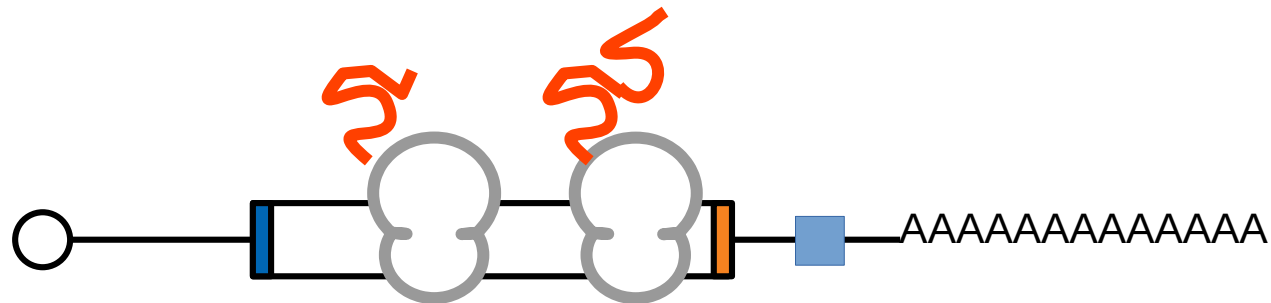
Main topic:

What role the poly(A) tail and cap play in mRNA degradation in yeast ?



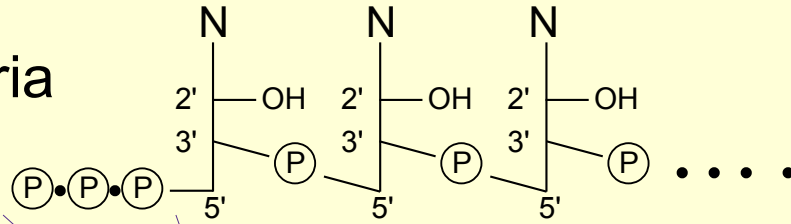
The Poly(A) Tail of mRNAs: Bodyguard in Eukaryotes, Scavenger in Bacteria

Marc Dreyfus^{1,3} and Philippe Régnier²

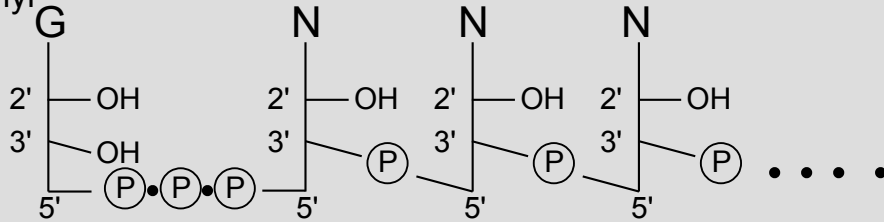


Protective structures at the 5' end (cap or 3P)

bacteria



7-methyl

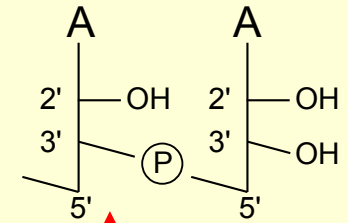
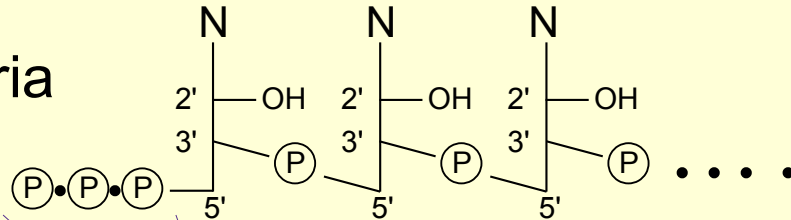


RNA cap

eukaryotes

poly(A) tails as mRNA protectors and inducers of RNA degradation

bacteria



AA

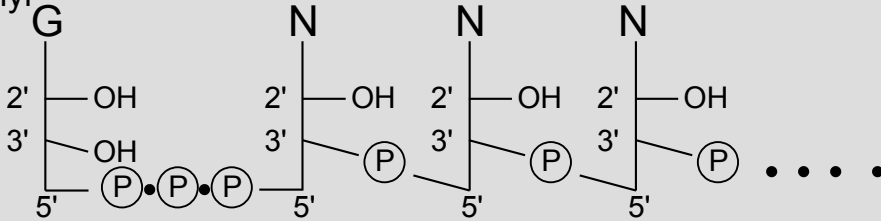
poly(A) tail

AAAAAAAAA

AAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAA

A few to hundreds of A

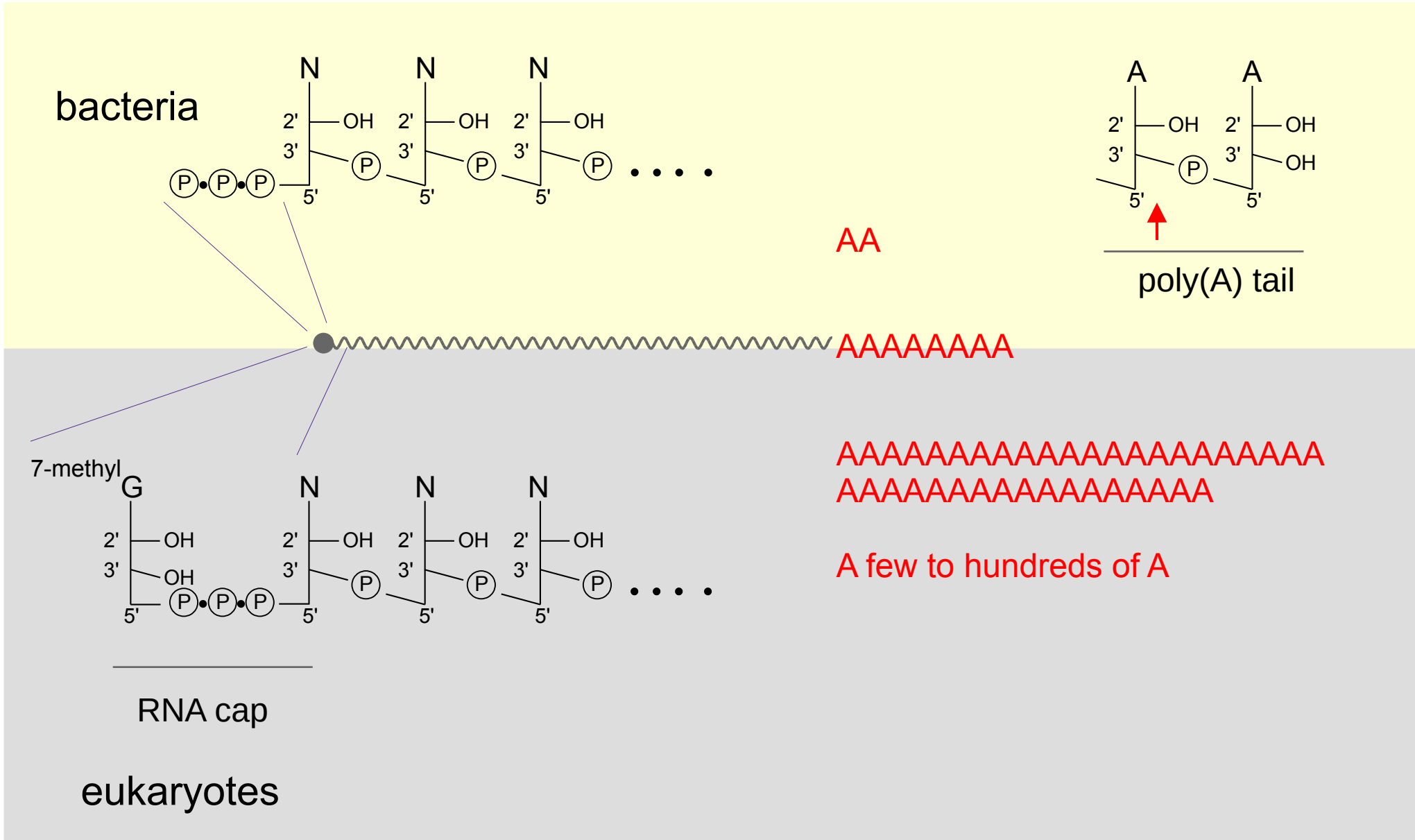
7-methyl



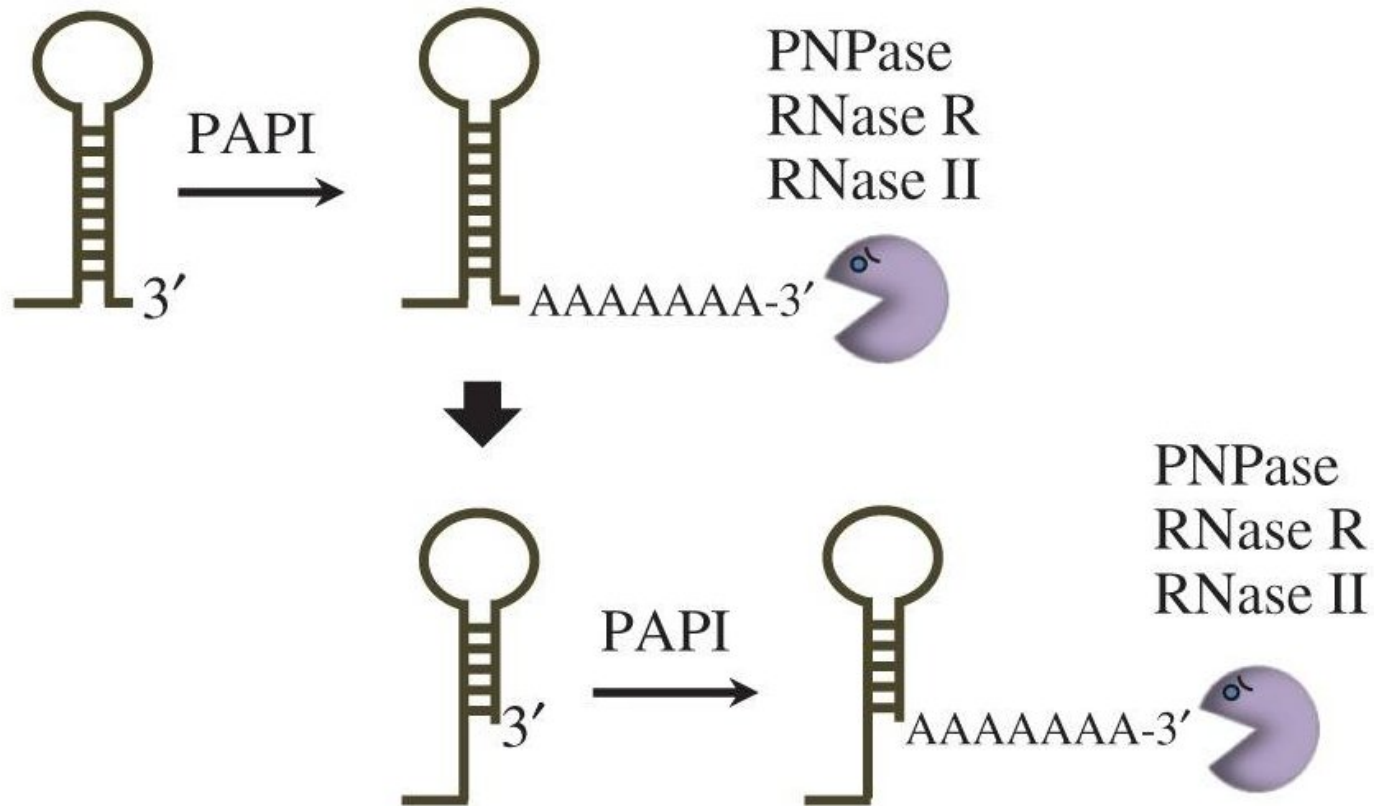
RNA cap

eukaryotes

Why the 3' end of RNAs gets a poly(A) for degradation ?



poly(A) can give a 'helping hand' to 3' to 5' exonucleases



The poly(A) is ancient

Poly(A)-**assisted** RNA degradation vs poly(A)-**protection**

bacteria
Archaea
organelles
nucleus

cytoplasm

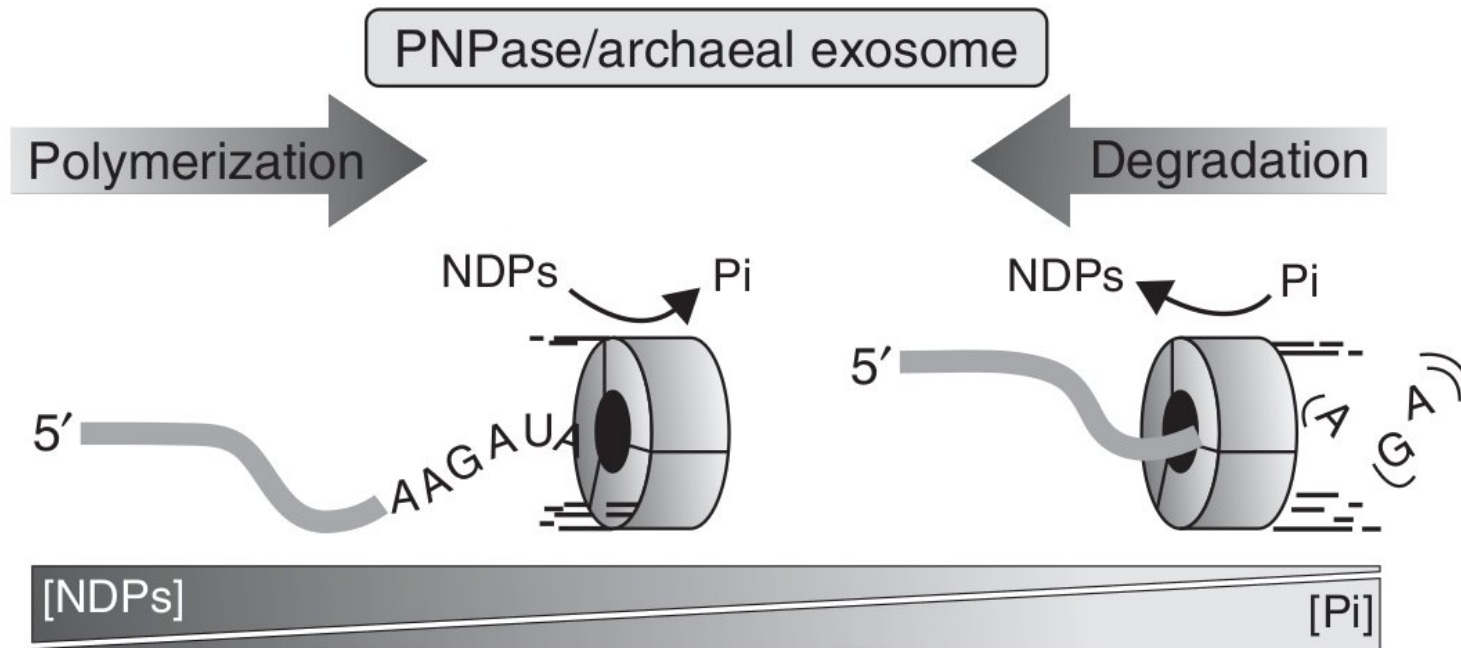
Cell, Vol. 111, 611–613, November 27, 2002, Copyright ©2002 by Cell

The Poly(A) Tail of mRNAs: Bodyguard in Eukaryotes, Scavenger in Bacteria

Marc Dreyfus^{1,3} and Philippe Régnier²

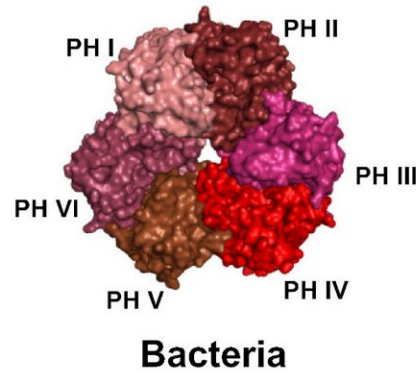
Evolutionary origin of the poly(A) tail ?

The **reversible** action of an exonuclease ?

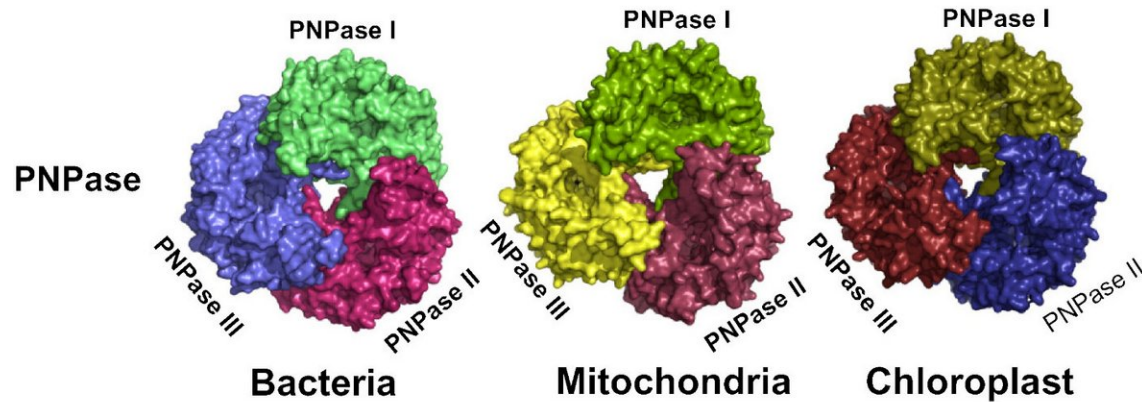


Multimeric 3' to 5' exonucleases (exosome)

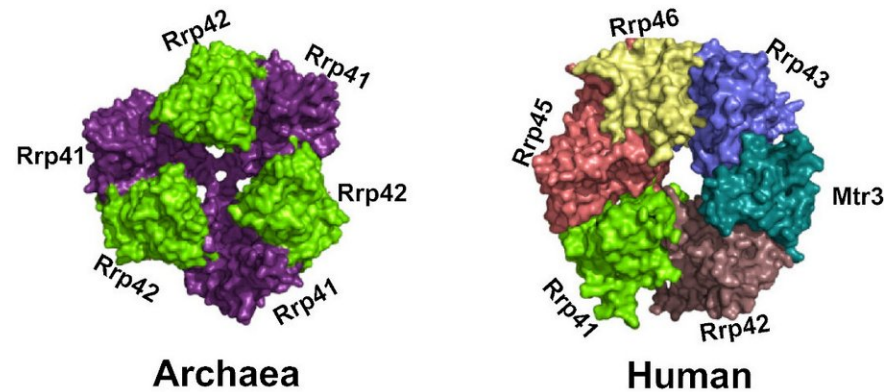
RNase PH



Polynucleotide
phosphorylase
PNPase



Exosome
core

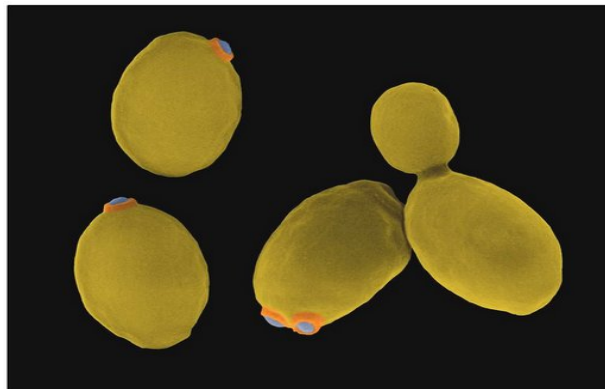


The discovery of the eukaryotic exosome was done in yeast

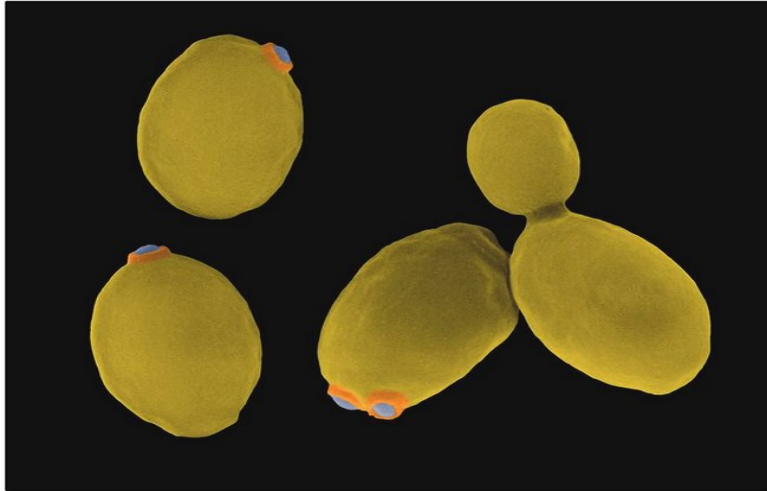
Cell, Vol. 91, 457–466, November 14, 1997, Copyright ©1997 by Cell Press

The Exosome: A Conserved Eukaryotic RNA Processing Complex Containing Multiple 3'→5' Exoribonucleases

**Philip Mitchell,^{*†} Elisabeth Petfalski,^{*}
Andrej Shevchenko, Matthias Mann,
and David Tollervey^{*}**



Dennis Kunkel, coloured SEM

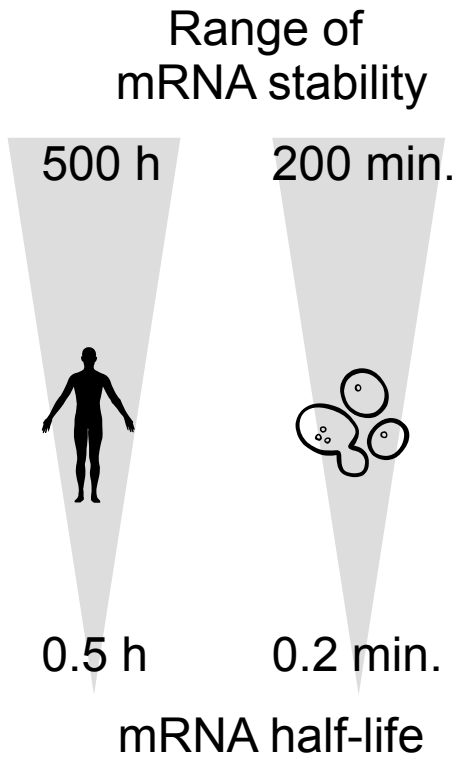


Dennis Kunkel, coloured SEM

A new role of poly(A) in **RNA degradation** in eukaryotes (2005, 2008, 2015, 2021)

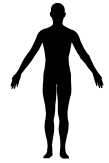
Is poly(A) length a marker of **mRNA instability** ? (2024)

mRNA instability can be crucial for life



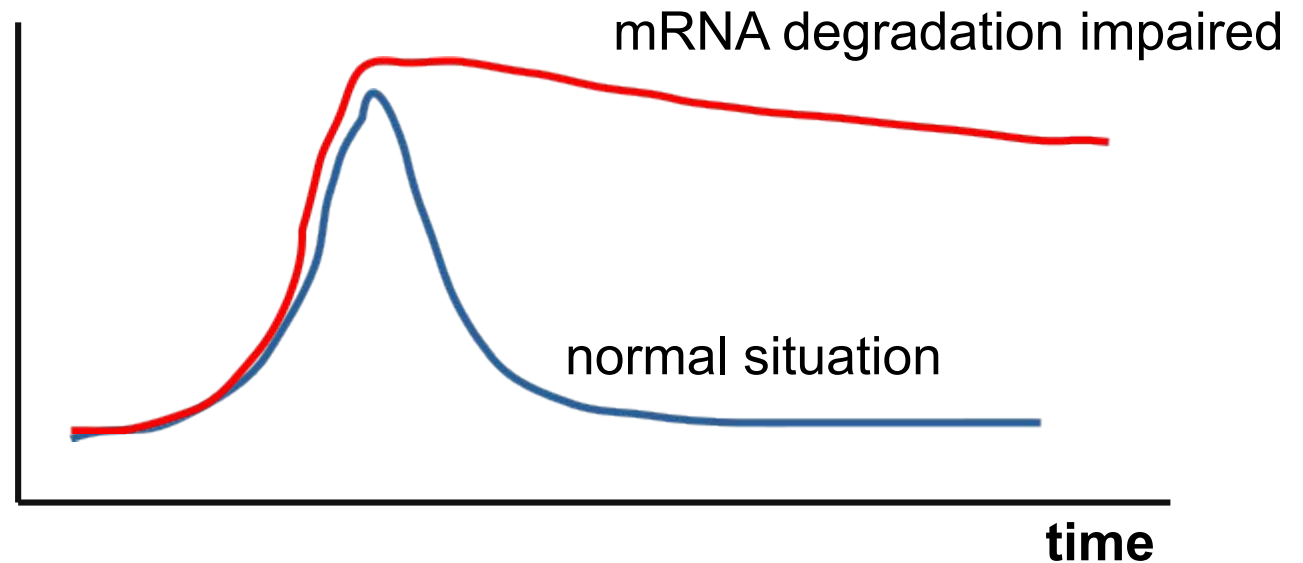
TNF- α mRNA stabilisation

→ hyperinflammation

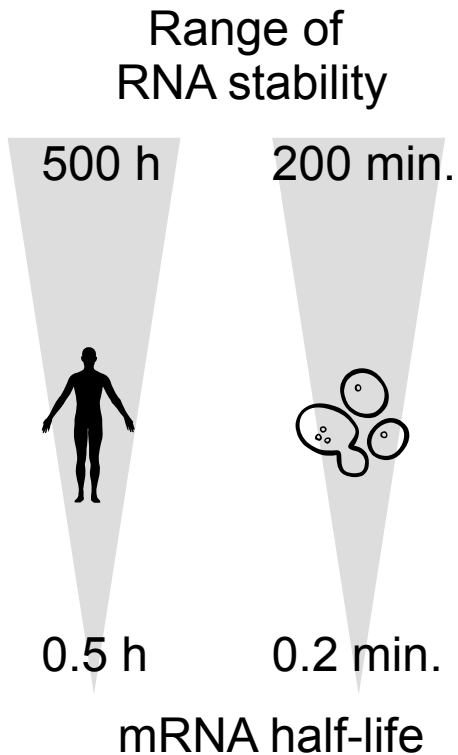


“Too much of a good thing”

TNF- α levels

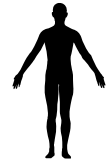


mRNA instability can be crucial for life

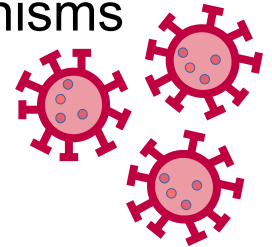


TNF- α mRNA stabilisation

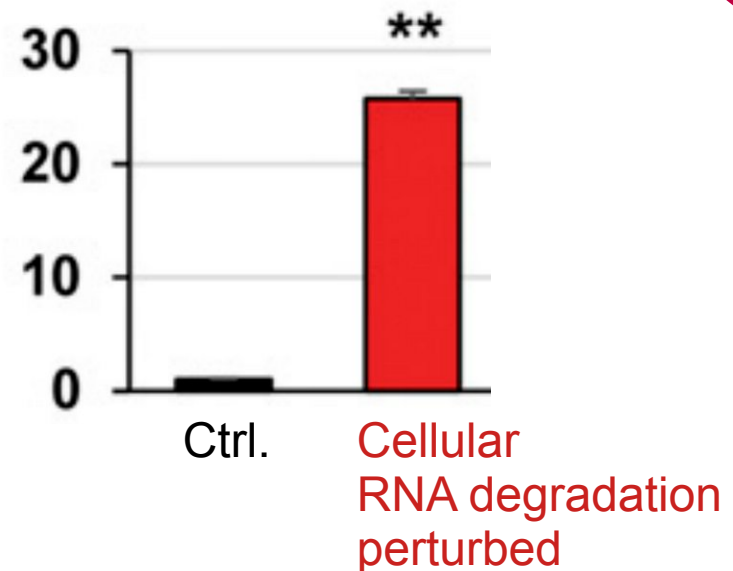
→ hyperinflammation



Viral mRNA → degraded by host mechanisms

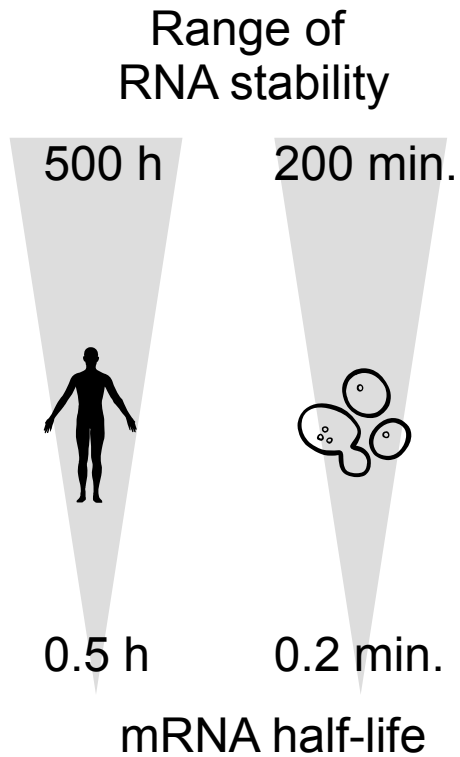


Fold increase in the number of coronavirus infected cells



Wada et al. *PNAS* 2018

mRNA instability can be crucial for life

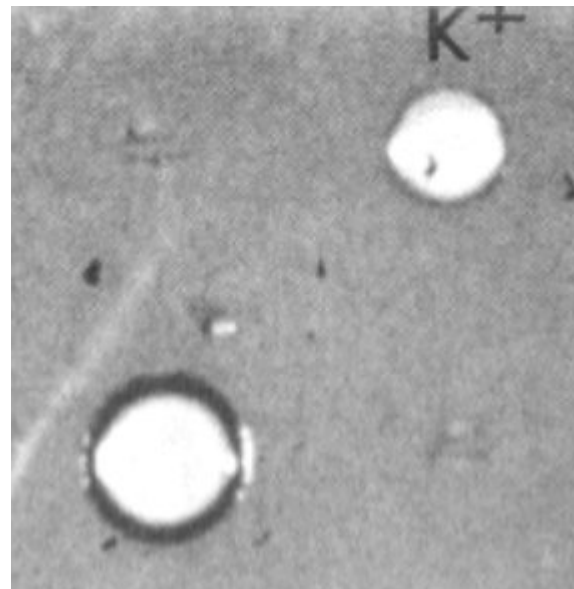
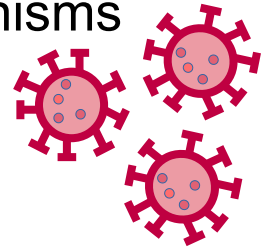


TNF- α mRNA stabilisation

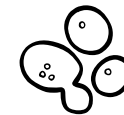
→ hyperinflammation



Viral mRNA → degraded by host mechanisms



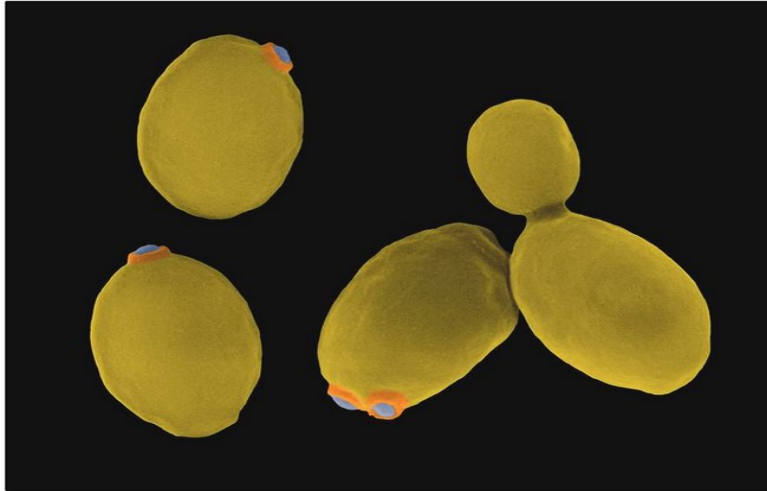
Killer yeast strain



Produces a toxin with the help of a defective virus

Superkiller yeast mutant (*ski2*)

Toh-E...Wickner, *J Bact* 1978



Dennis Kunkel, coloured SEM

A new role of poly(A) in **RNA degradation** in eukaryotes (2005, 2008, 2015, 2021)

Is poly(A) length a marker of **mRNA instability** ? (2024)

Nucleus

Exosome+

Rrp6

Mpp6

Mtr4...

Cytoplasm

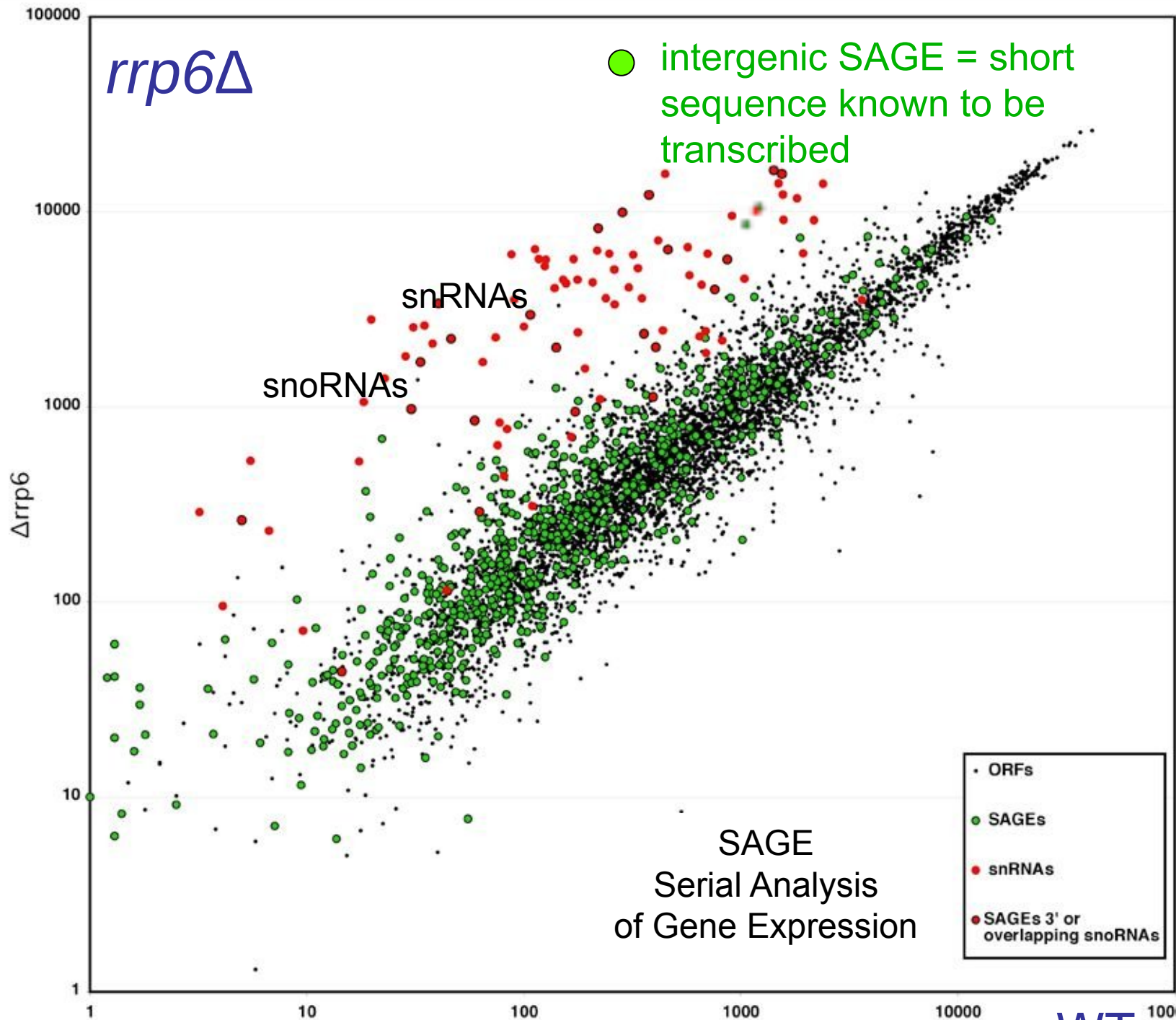
The major 3' to 5' RNA
degradation activities
in yeast

Exosome+
Ski complex

Problem: How to study
the function of RRP6
(and nuclear exosome) ?

rrp6 Δ

● intergenic SAGE = short sequence known to be transcribed



100000

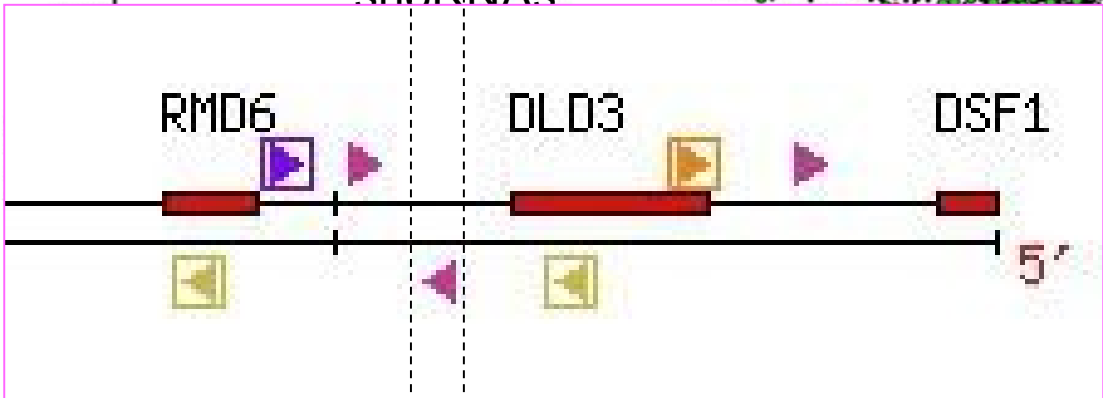
rrp6Δ

● intergenic SAGE = short sequence known to be transcribed

10000

snRNAs

snoRNAs



10

SAGE
Serial Analysis
of Gene Expression

- ORFs
- SAGEs
- snRNAs
- SAGEs 3' or overlapping snoRNAs

1

10

100

1000

10000

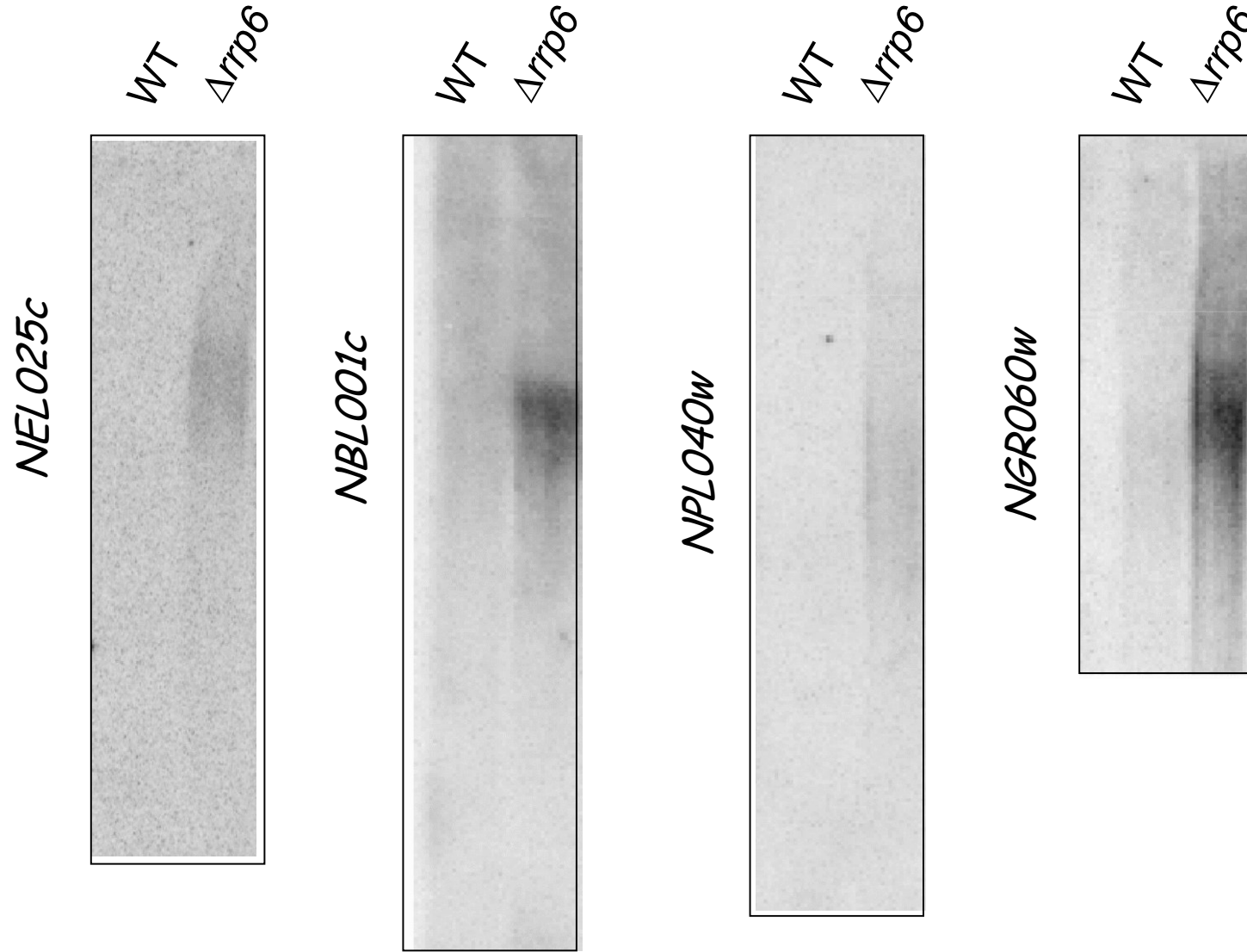
100000

WT

WT

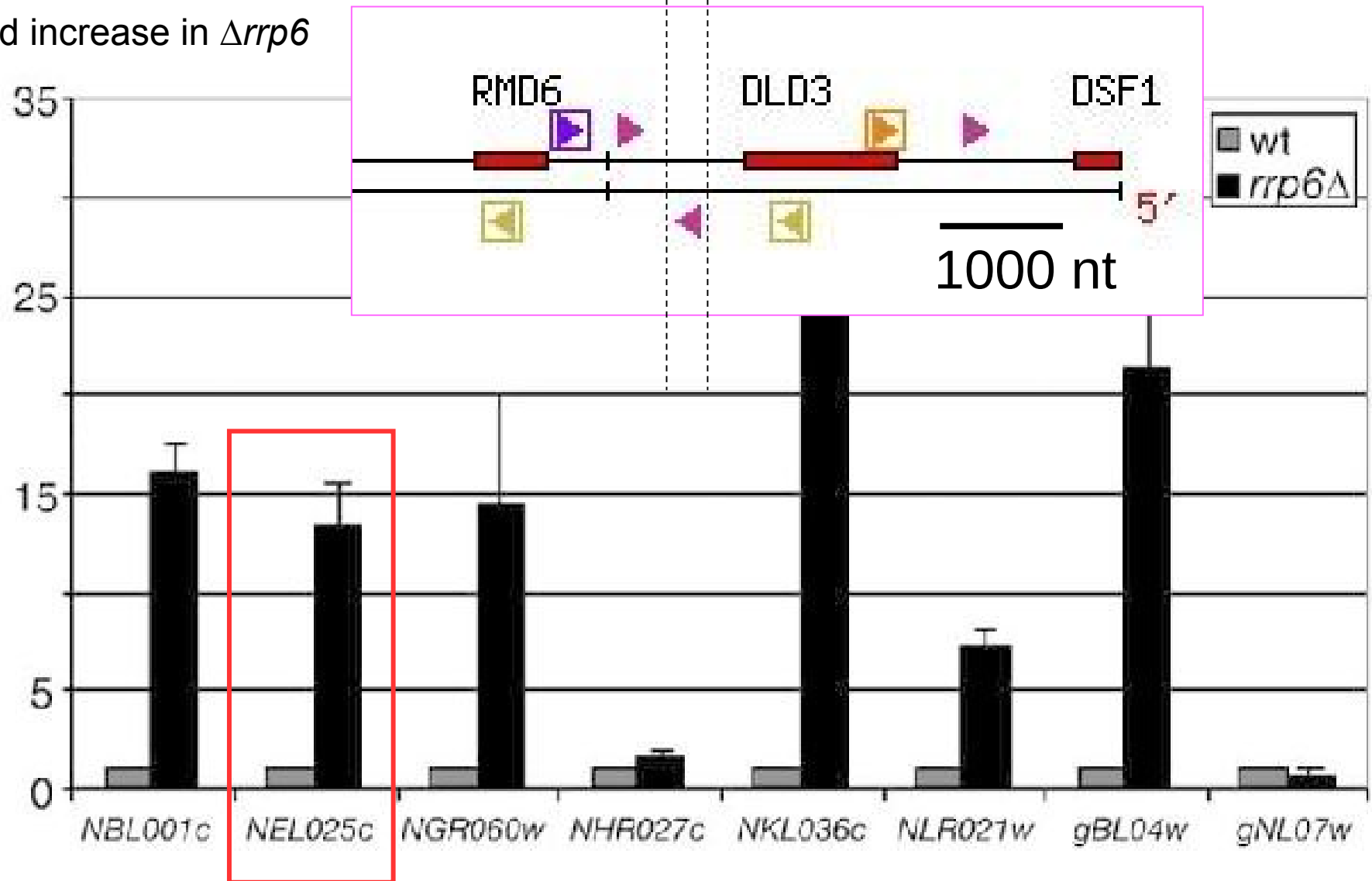
Problem: Do these transcripts exist ?

Intergenic regions are expressed in *rrp6* Δ

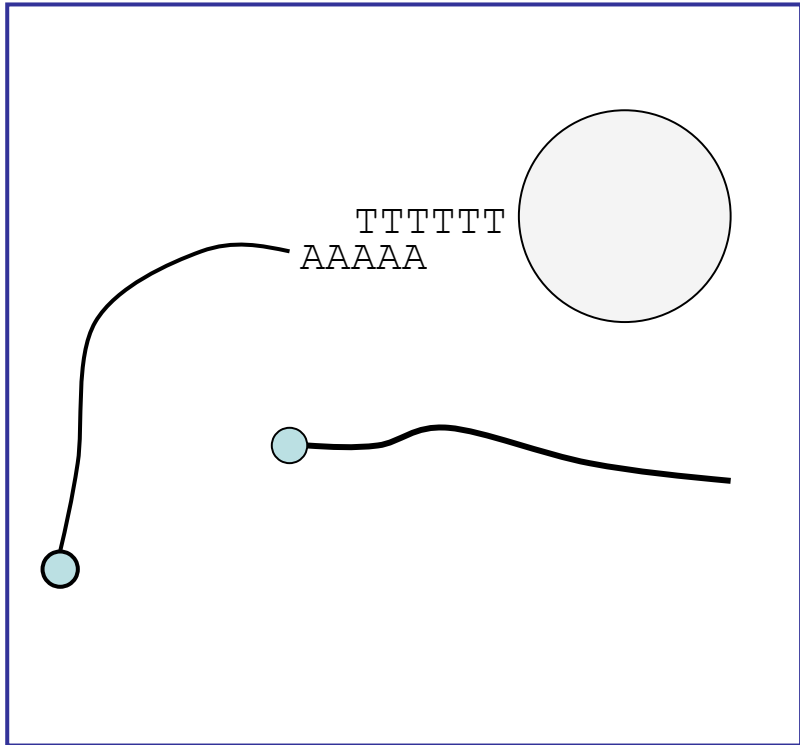


Quantitative RT-PCR confirms transcription from intergenic regions

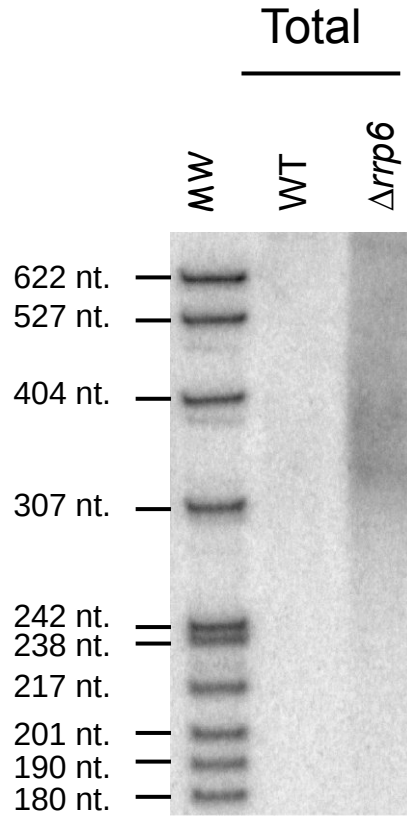
Fold increase in $\Delta rrp6$



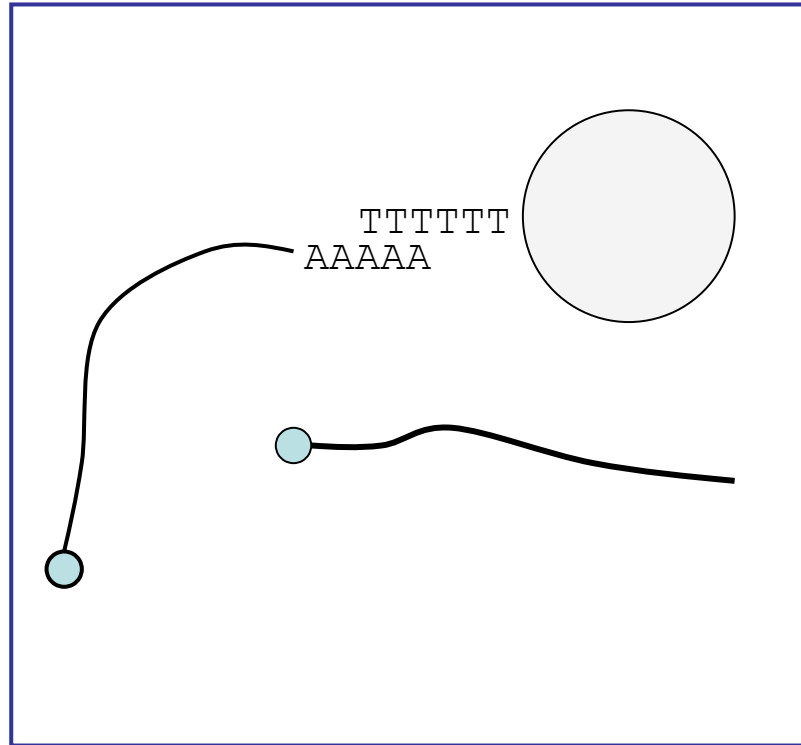
Problem: Are the
intergenic transcripts
poly-Adenylated ?



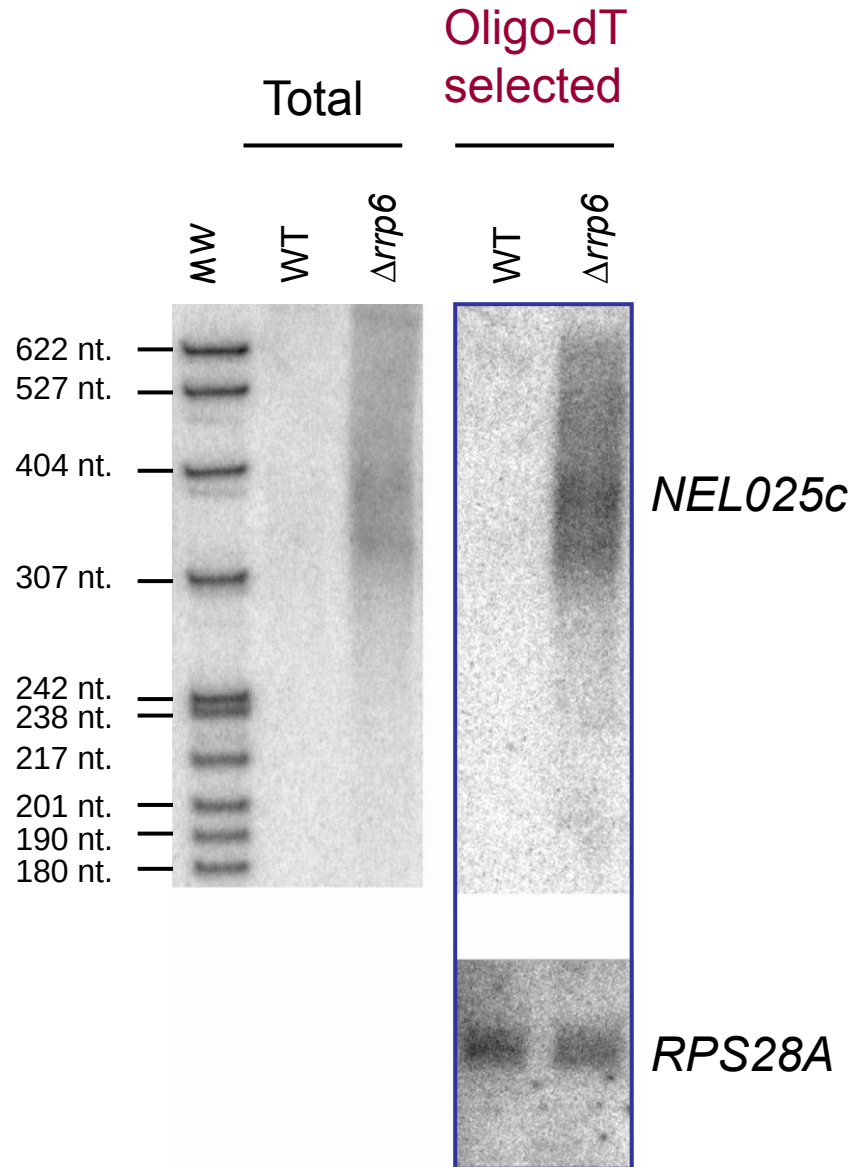
Oligo-dT chromatography to select poly(A) RNAs



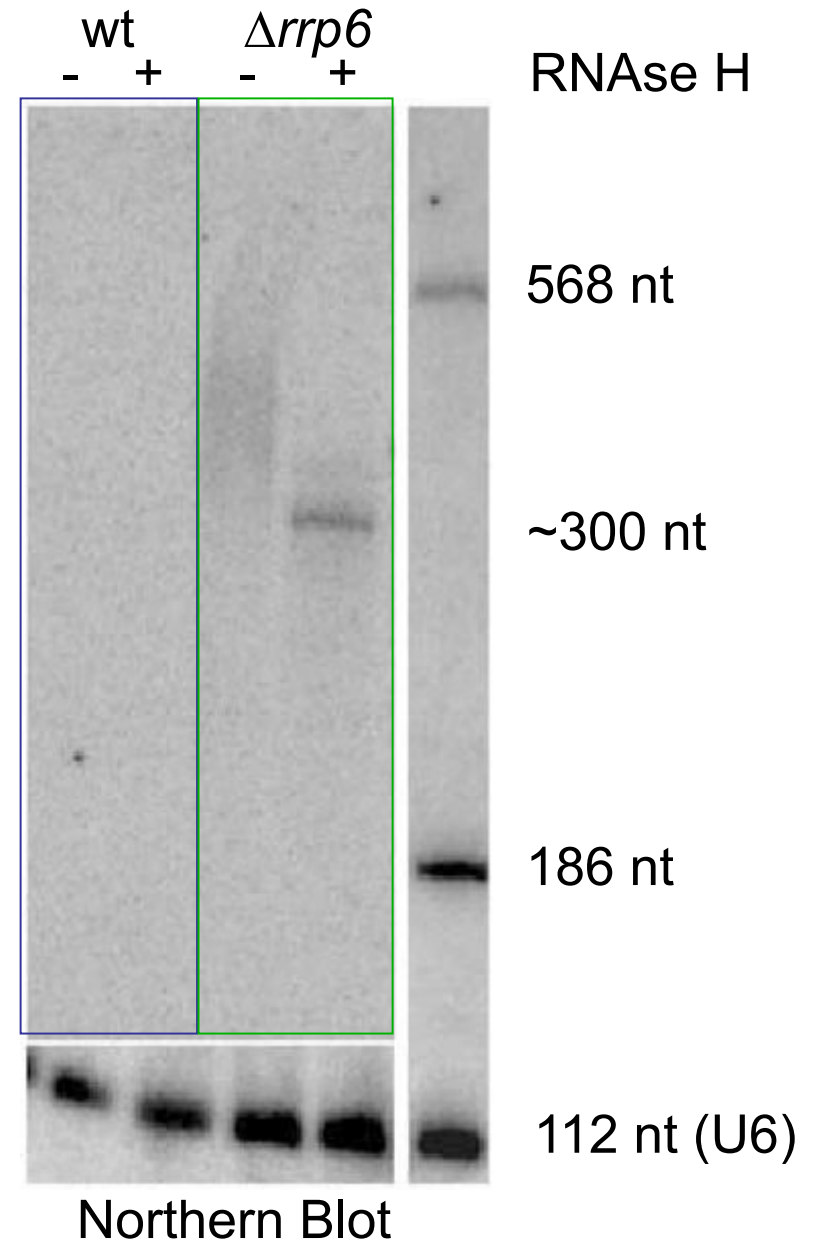
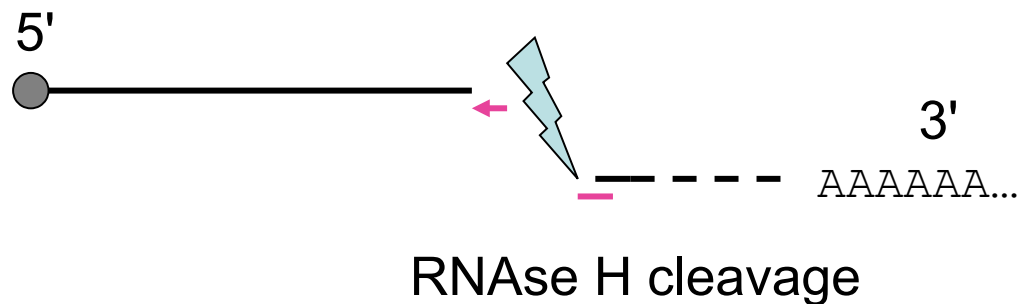
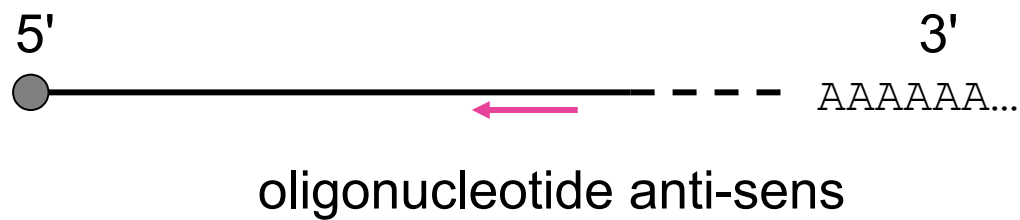
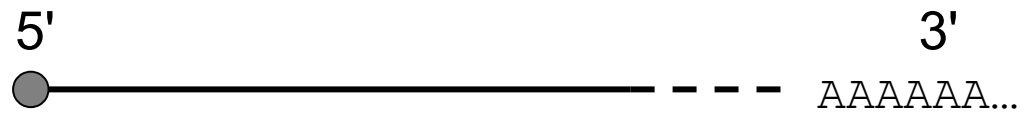
NEL025c transcripts are polyadenylated in *rrp6* Δ



Oligo-dT chromatography to select poly(A) RNAs



NEL025c transcripts have a defined capped 5' end

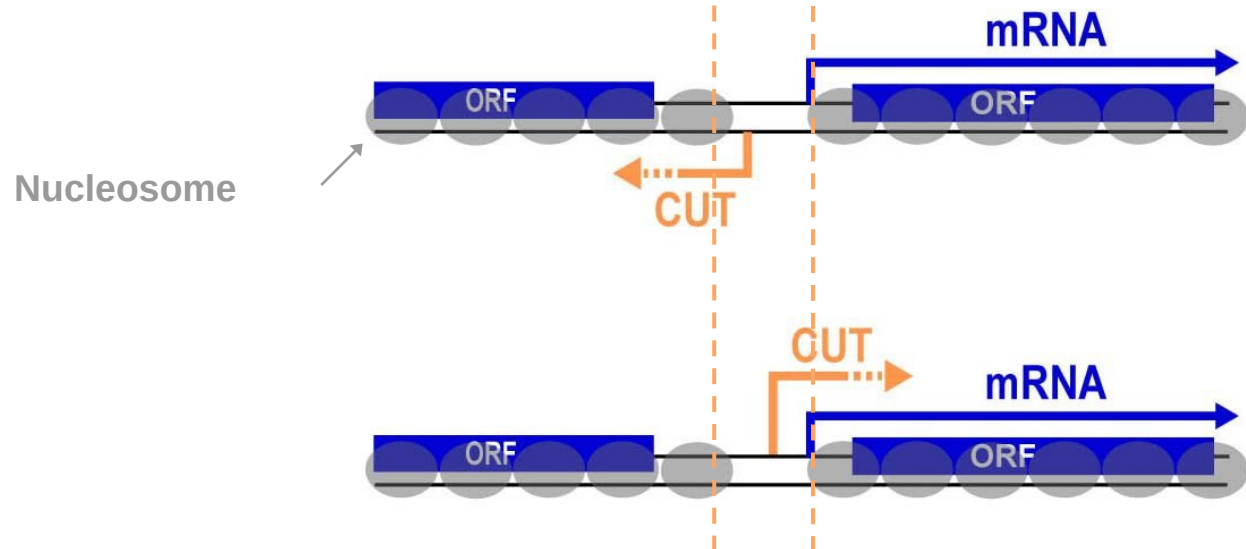
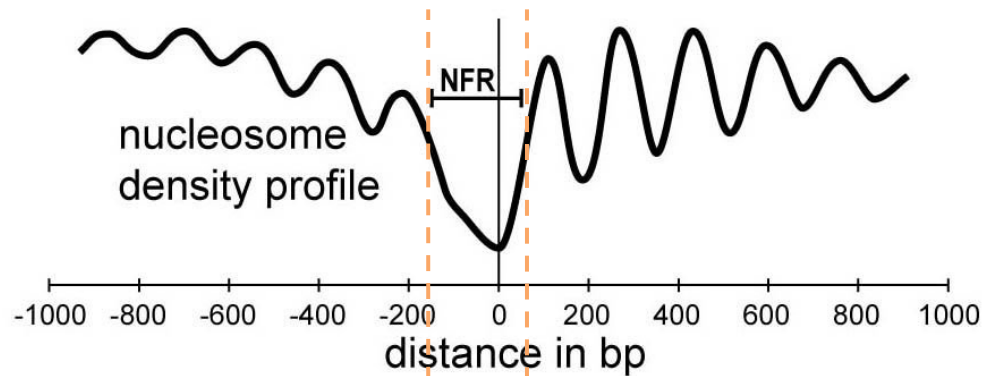


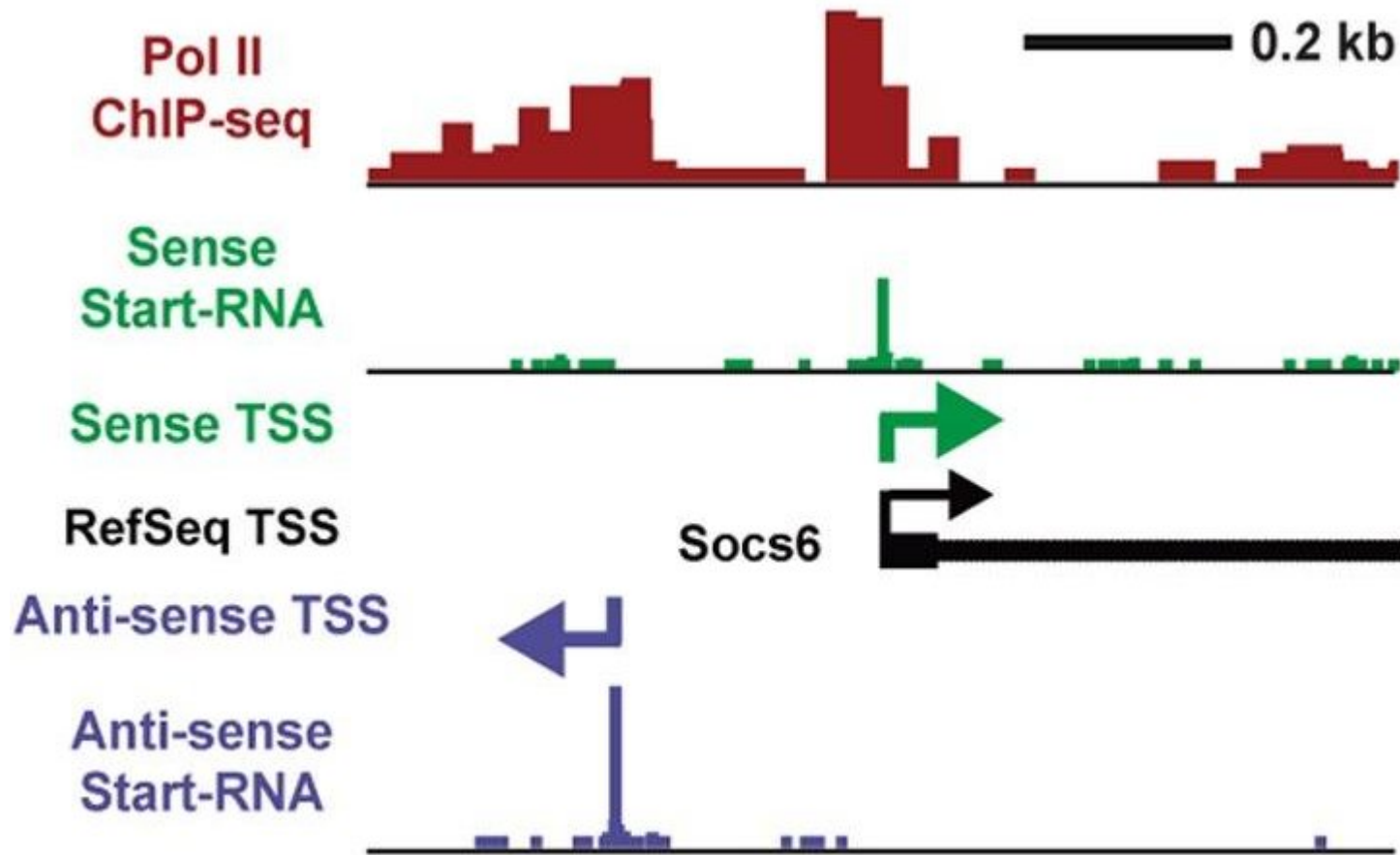
Problem: Why the levels
of NEL025 increase ?

Cryptic Unstable Transcripts (CUTs)

Associated with gene promoters

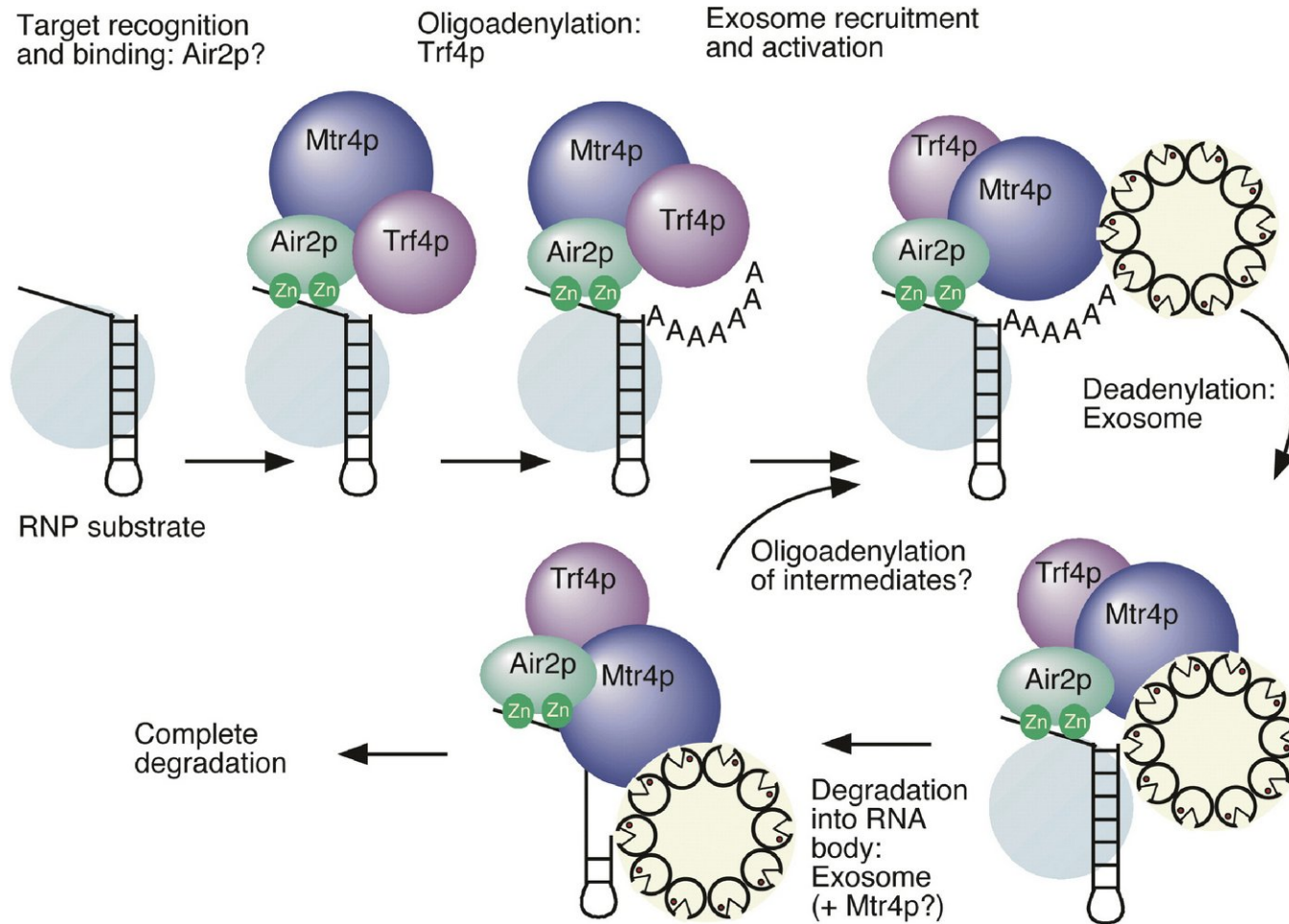
arise from Nucleosome Free Regions.

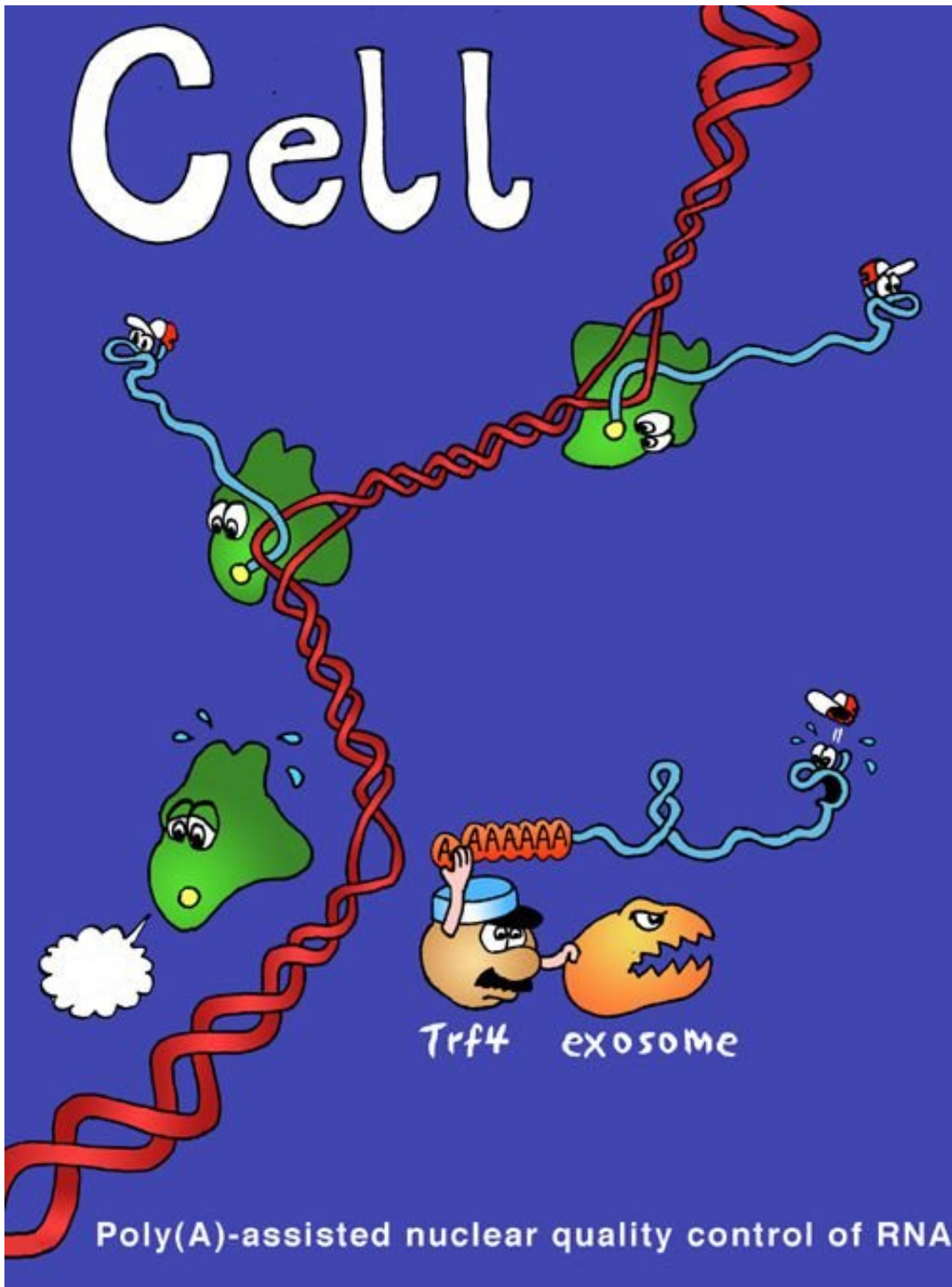




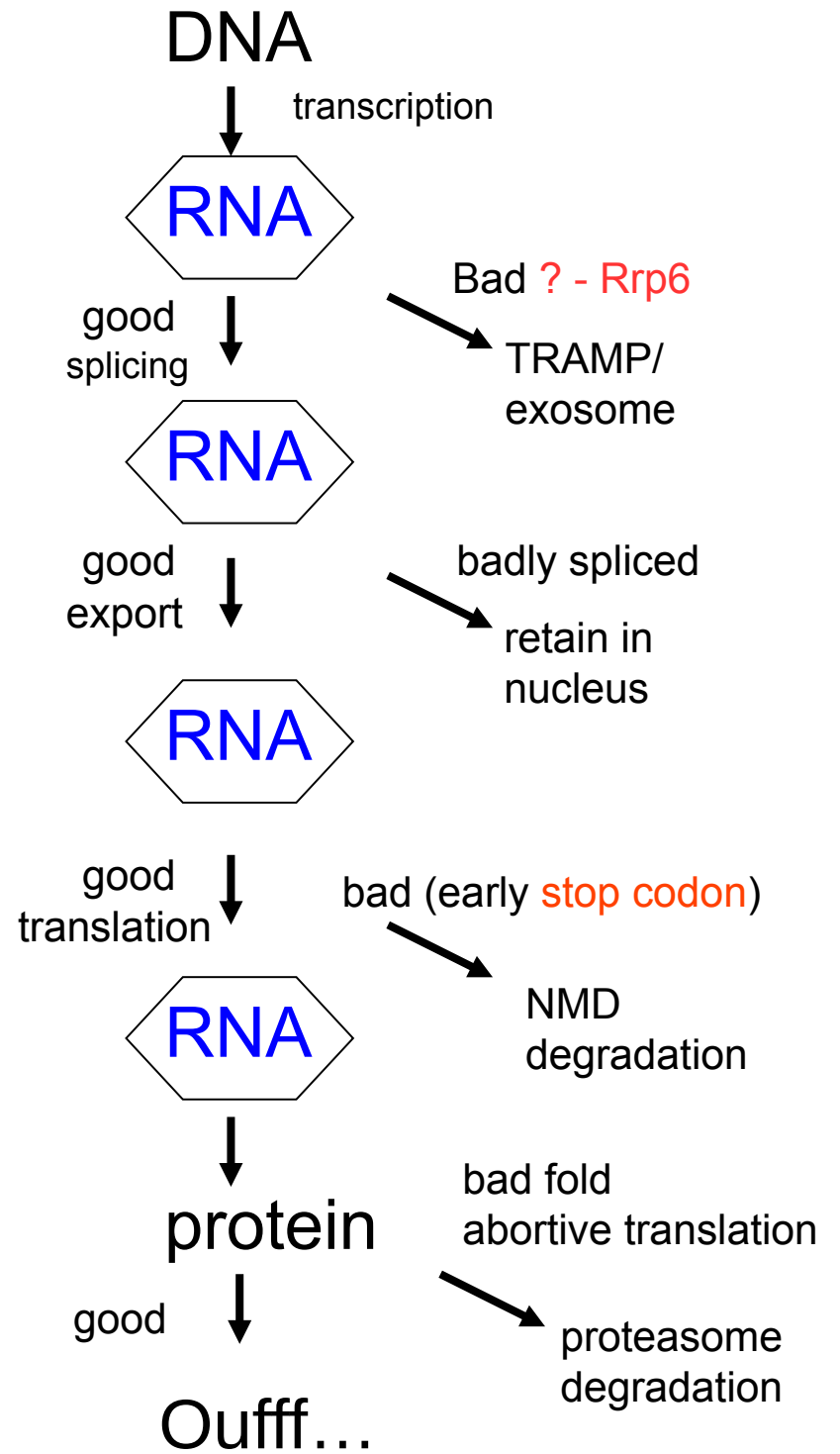
Example of divergent transcription in mammalian cells

Polyadenylation marks 3' ends to enhance degradation of pervasive transcripts

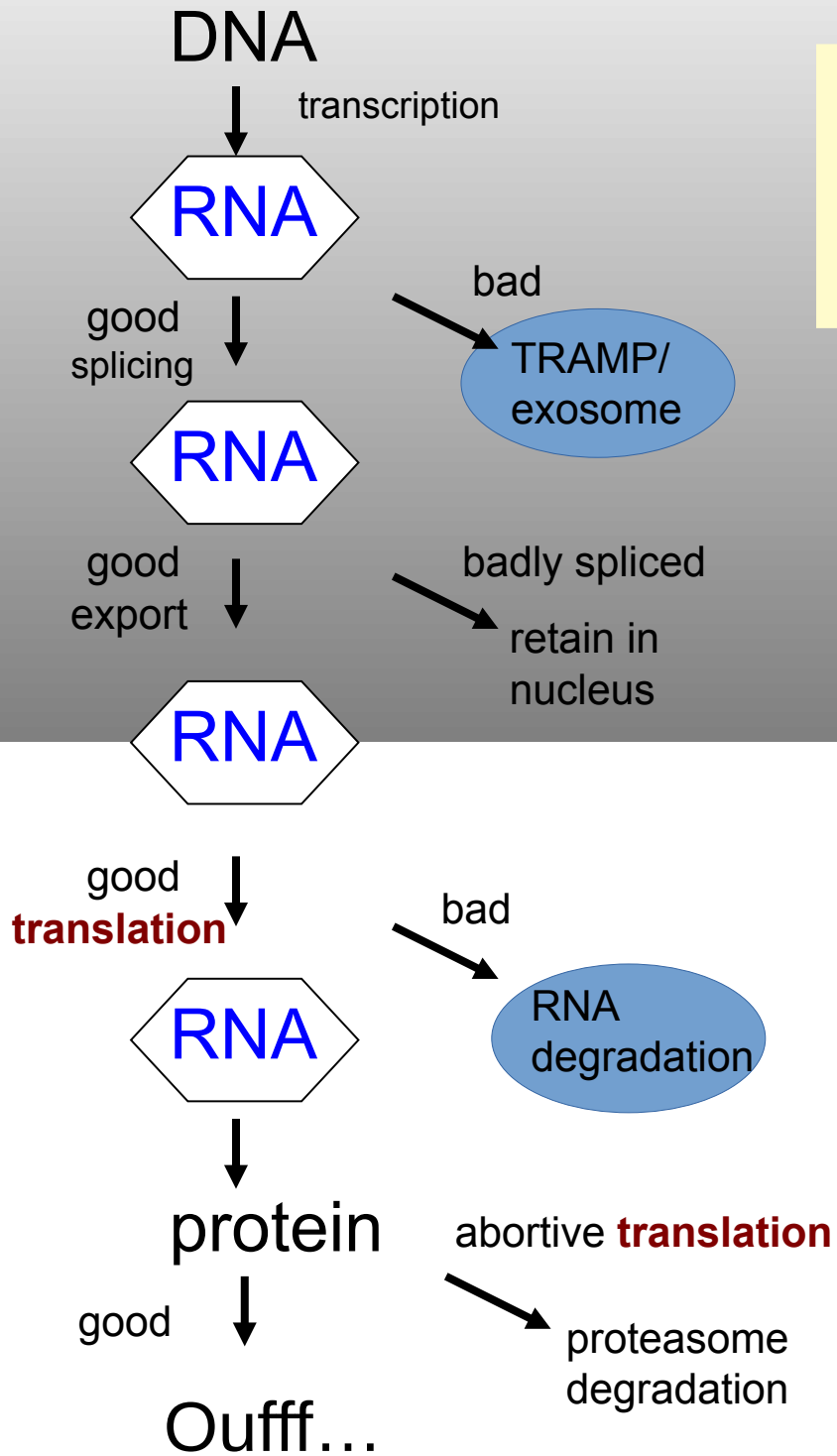


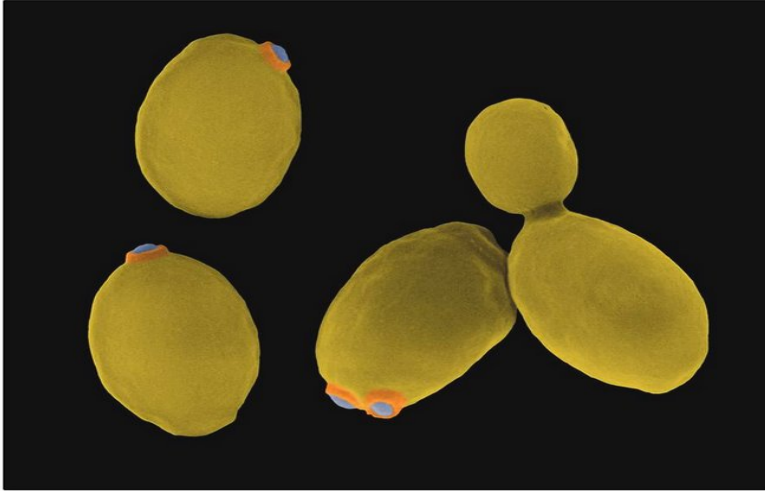


© Ebbe Sloth Andersen, University of Aarhus, Denmark 2005



"If anything can go wrong, it will"
Murphy's law or the fourth law of thermodynamics



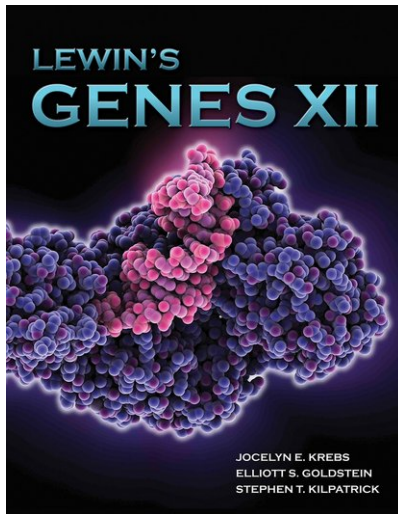


Dennis Kunkel, coloured SEM

A new role of poly(A) in **RNA degradation** in eukaryotes (2005, 2008, 2015, 2021)

Is poly(A) length a marker of **mRNA instability** ? (2024)

2017



poly[A] tail of mRNAs

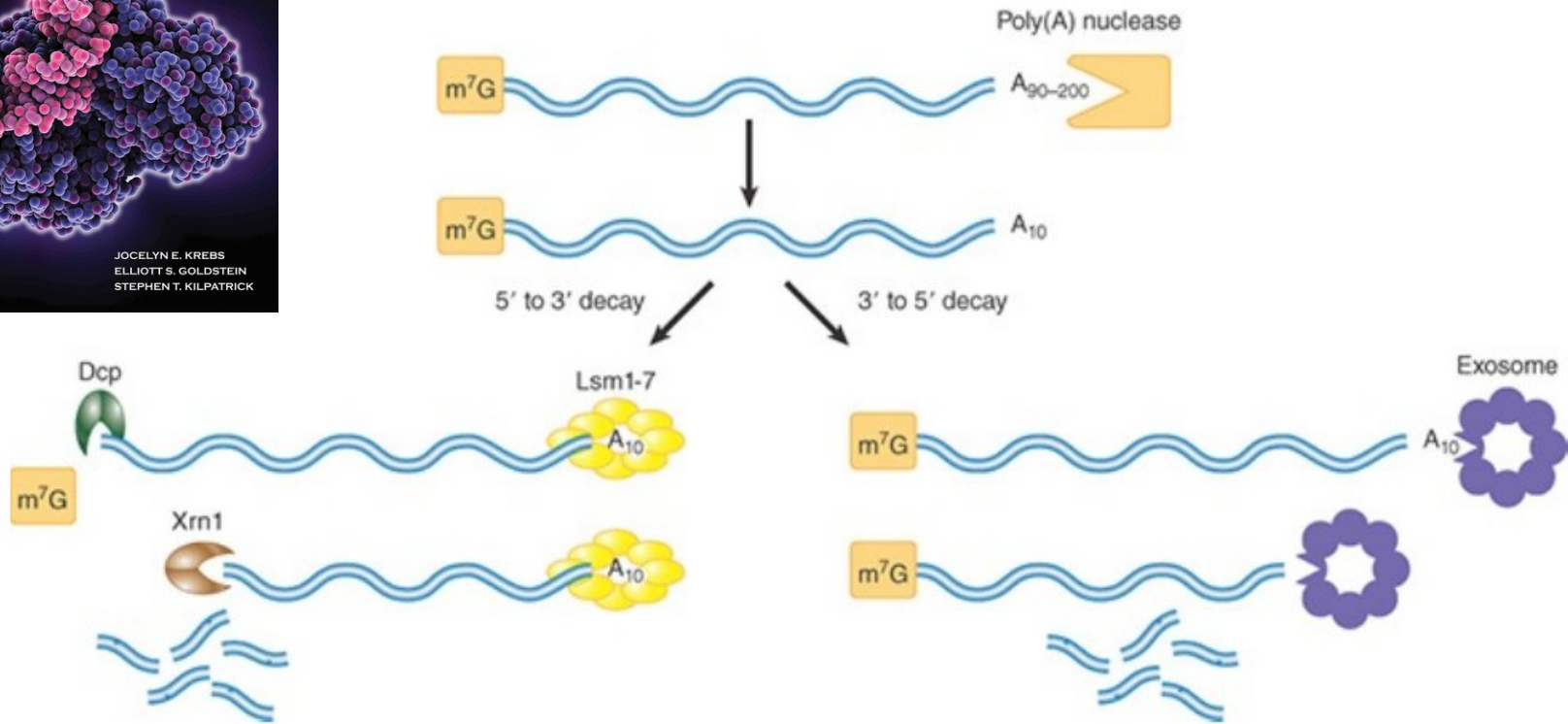
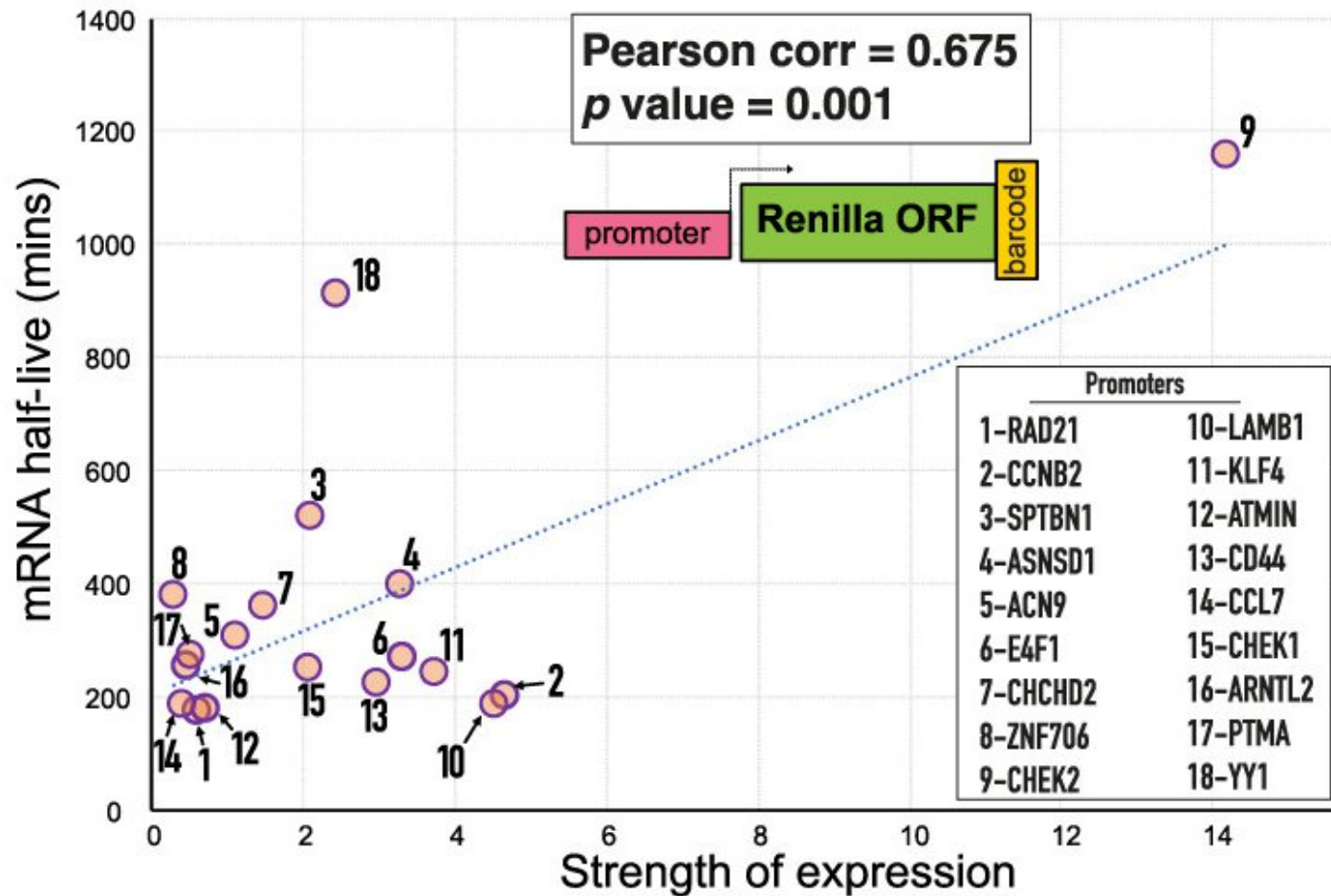


FIGURE 20.6 The major deadenylation-dependent decay pathways in eukaryotes. Two pathways are initiated by deadenylation.

“However, the reviewers also noted that the broader field of RNA decay pathways, and the roles of deadenylation and decapping in these pathways, **is relatively mature, with extensive existing datasets and models.**”

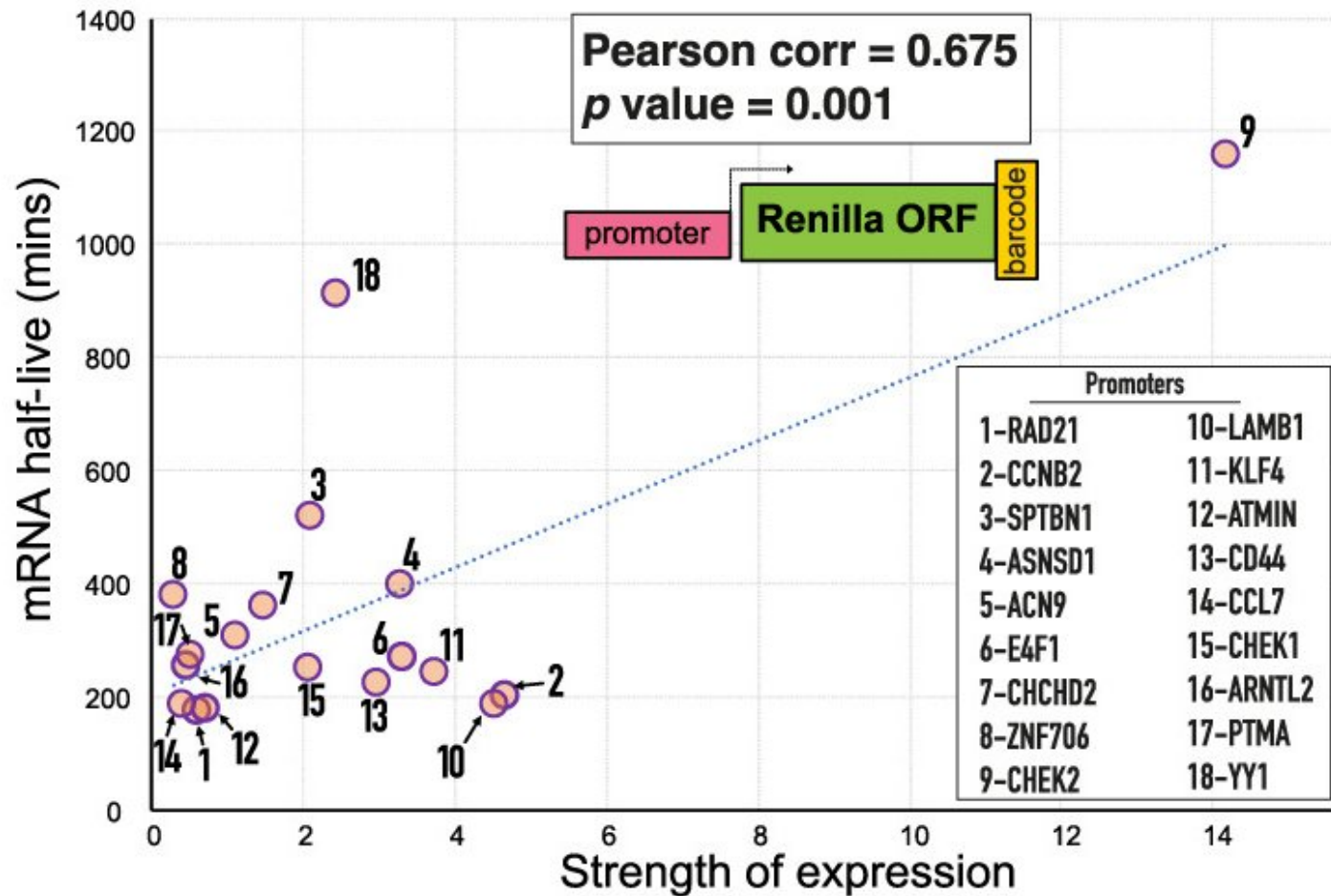
Letter of rejection from
a reputable journal editor
January 2024

Large scale results and conclusions in science...



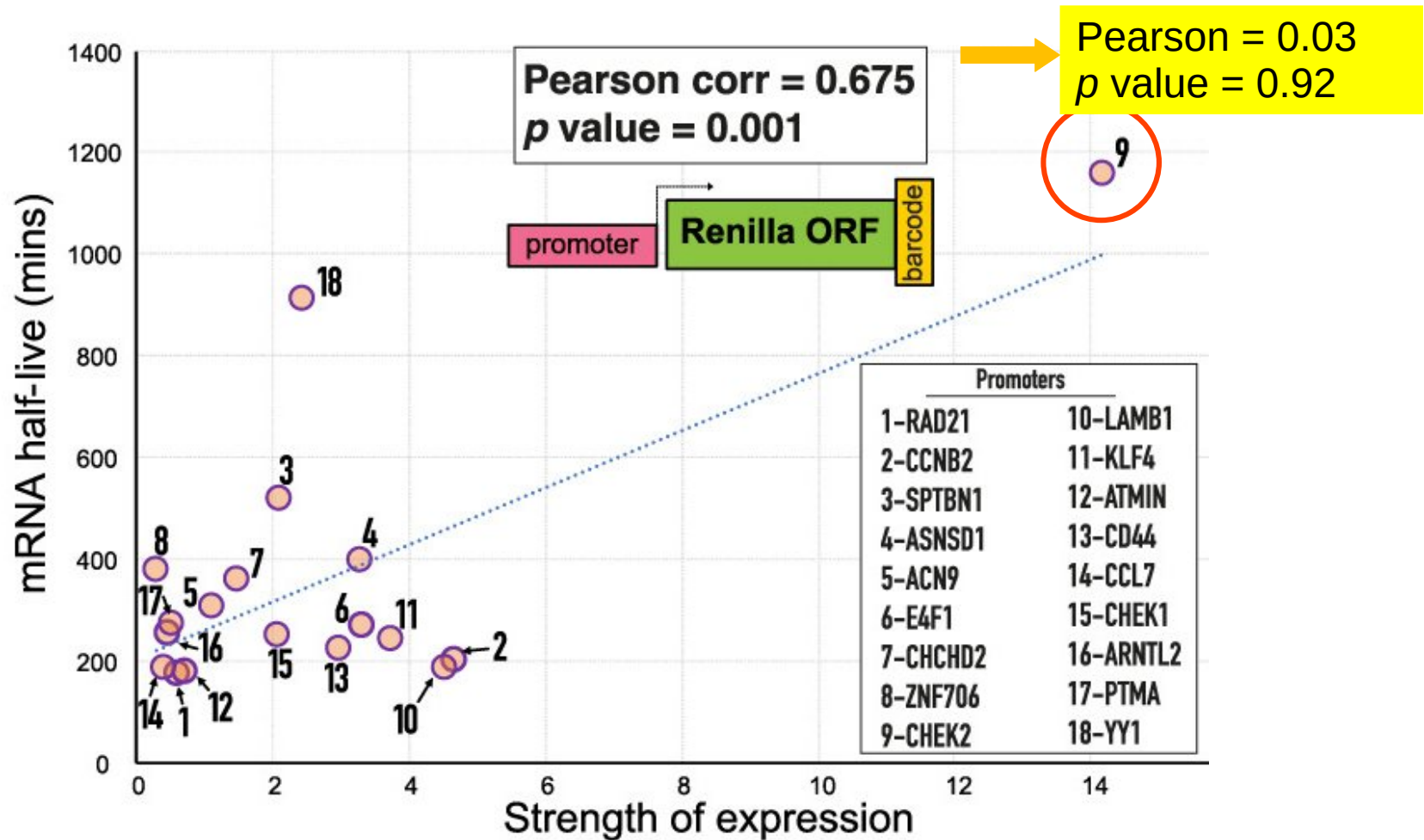
Slobodin et al., *Mol Cell* 2020
cited 65 times (as of 2024)

“ Interestingly, we observed a positive correlation ($r = 0.675$, $p = 0.001$; **Figure 1E**) between the promoter potency to drive mRNA expression and the stability of the respective transcripts, further confirming the positive effect of transcription on mRNA stability.”



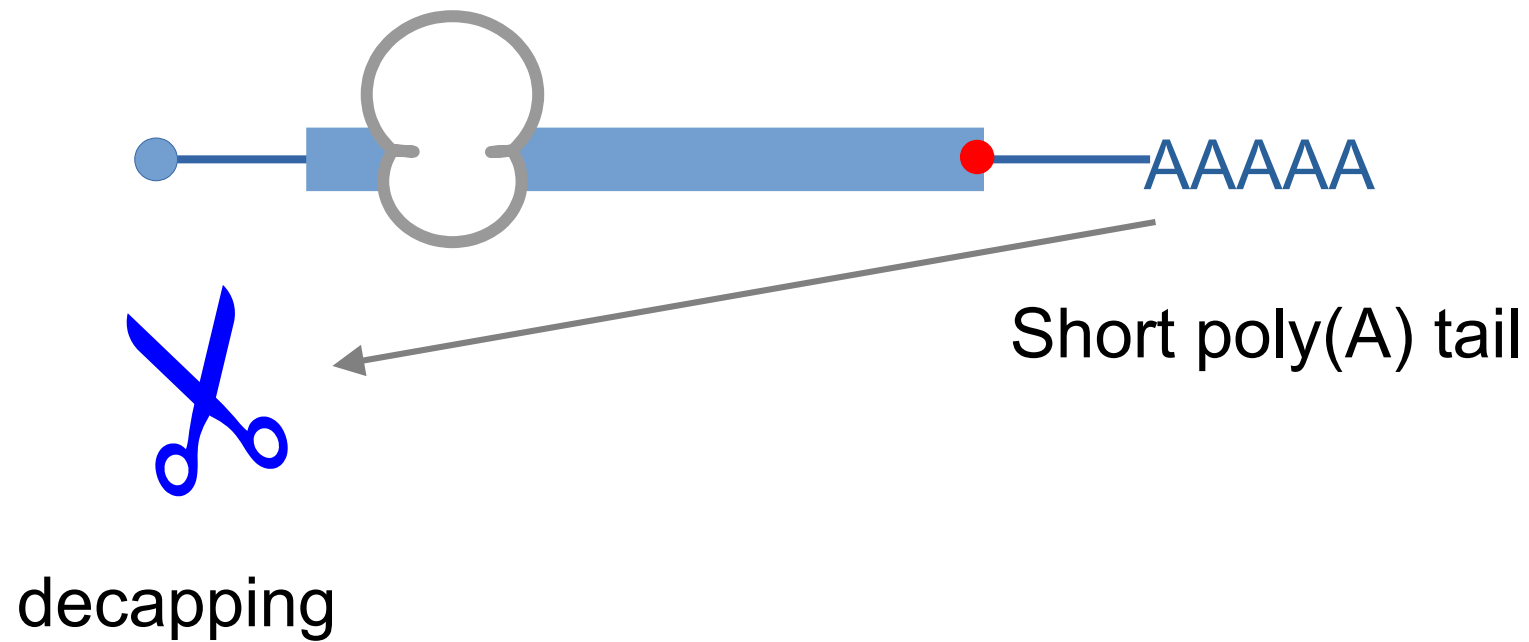
Slobodin et al., *Mol Cell* 2020
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“ Interestingly, we observed a positive correlation ($r = 0.675$, $p = 0.001$; **Figure 1E**) between the promoter potency to drive mRNA expression and the stability of the respective transcripts, further confirming the positive effect of transcription on mRNA stability.”

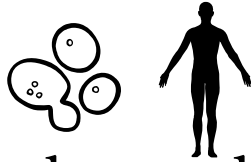


Slobodin et al., *Mol Cell* 2020
cited 67 times (as of 2024)

mRNA degradation in eukaryotes in textbooks



Current view:

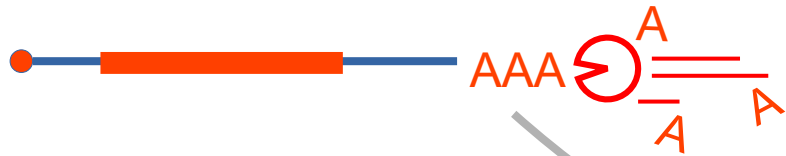


mRNA degradation depends on **deadenylation** speed

stable mRNA



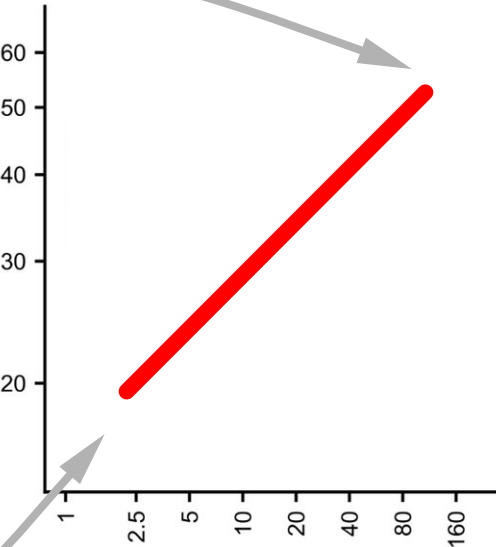
unstable mRNA



Length of poly(A) tail

long

short



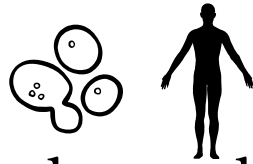
unstable

stable

mRNA half-life (min)

expected

Current view:



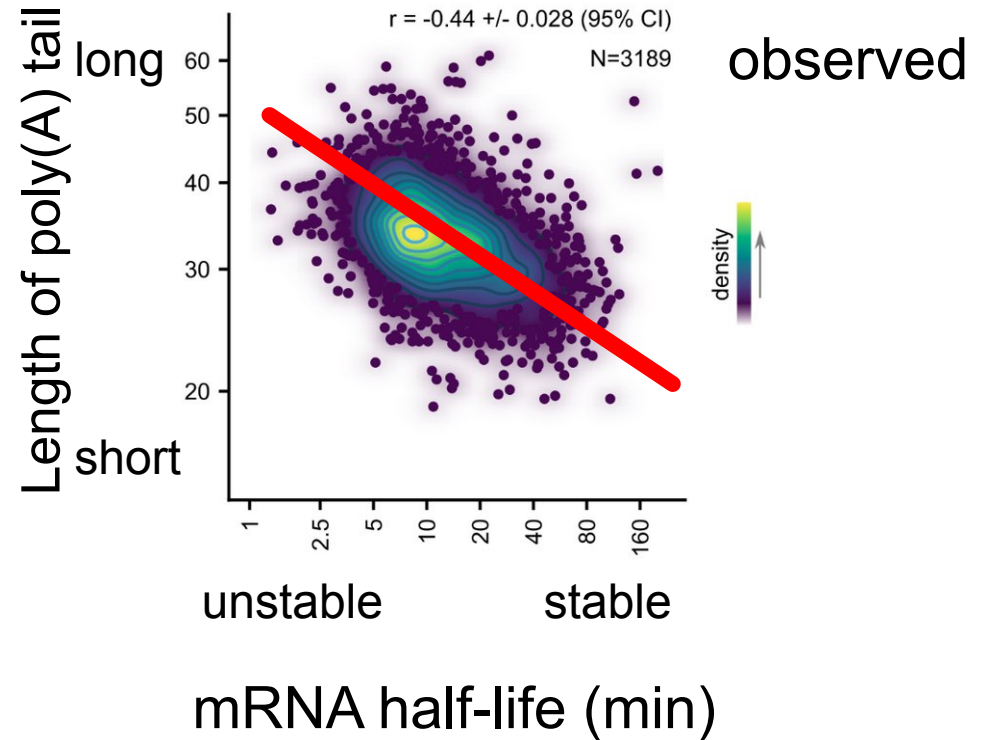
mRNA degradation depends on **deadenylation** speed

Incompatible with large-scale results!

stable mRNA



unstable mRNA



poly(A) length – Subtelny et al., *Nature* 2014
mRNA stability – Miller et al., *Mol Syst Biol* 2011

Long poly(A) tail = unstable mRNA ?

Lima et al., *NSMB* 2017



Short poly(A) tails are a conserved feature of highly expressed genes



Legnini et al., *Nature Methods*, 2019



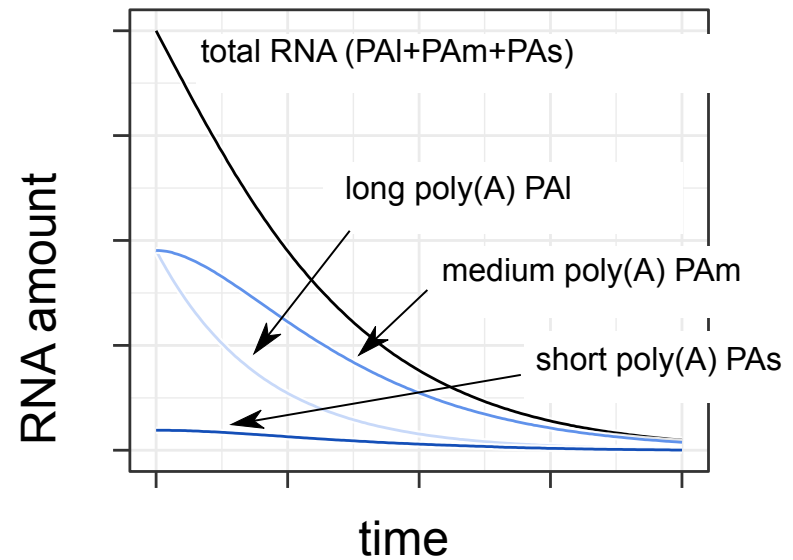
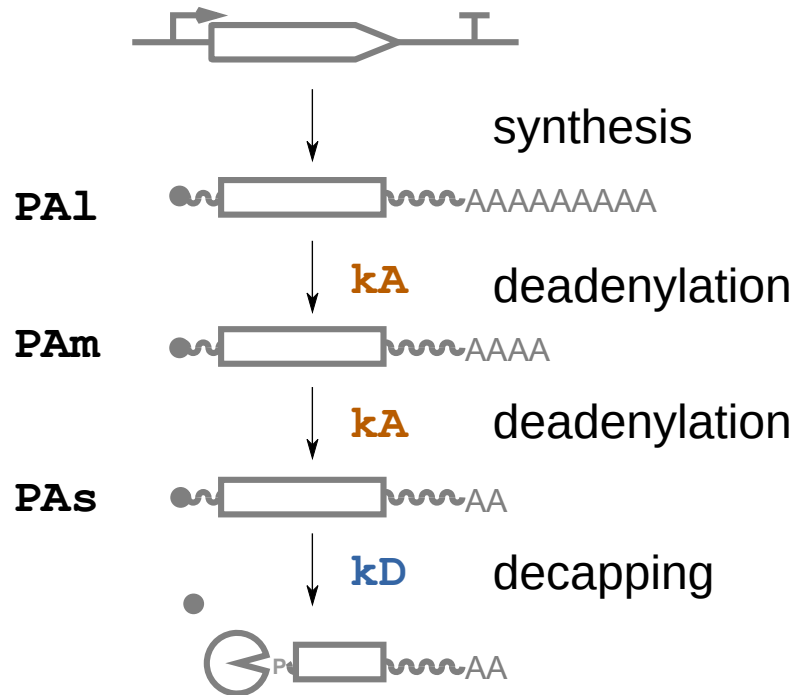
negative correlation
stability/poly(A) tail length

Jia et al., *Nature Plants*, 2022

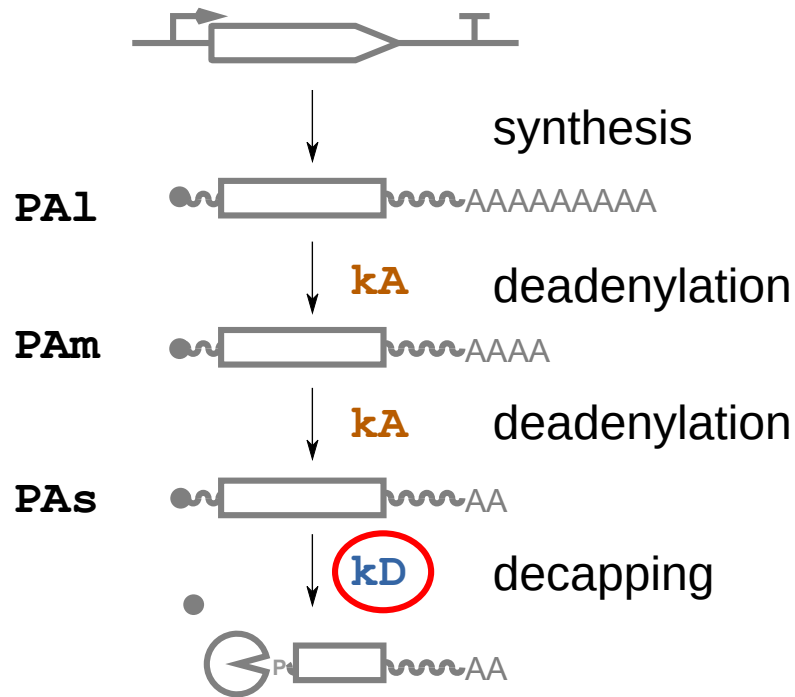


“mRNAs with short half-lives in general have long poly(A) tails, while mRNAs with long half-lives are featured with relatively short poly(A) tails”

Simulating mRNA degradation and poly(A) tails



Decapping rate variability can explain poly(A) tail distribution at steady state



Eisen et al., *Mol Cell* 2020



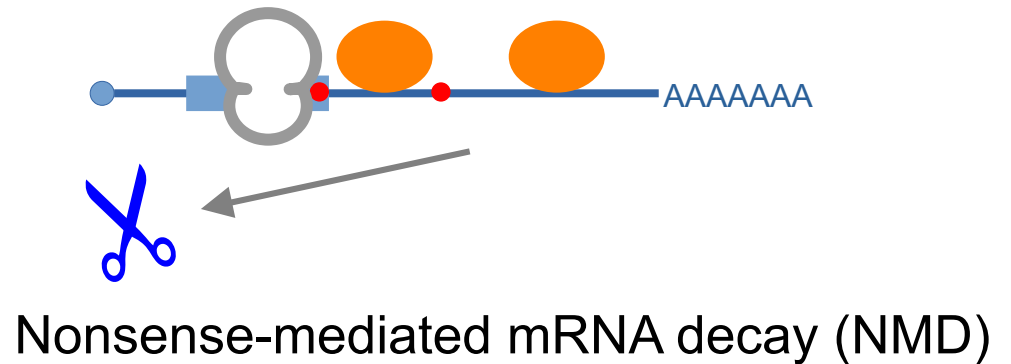
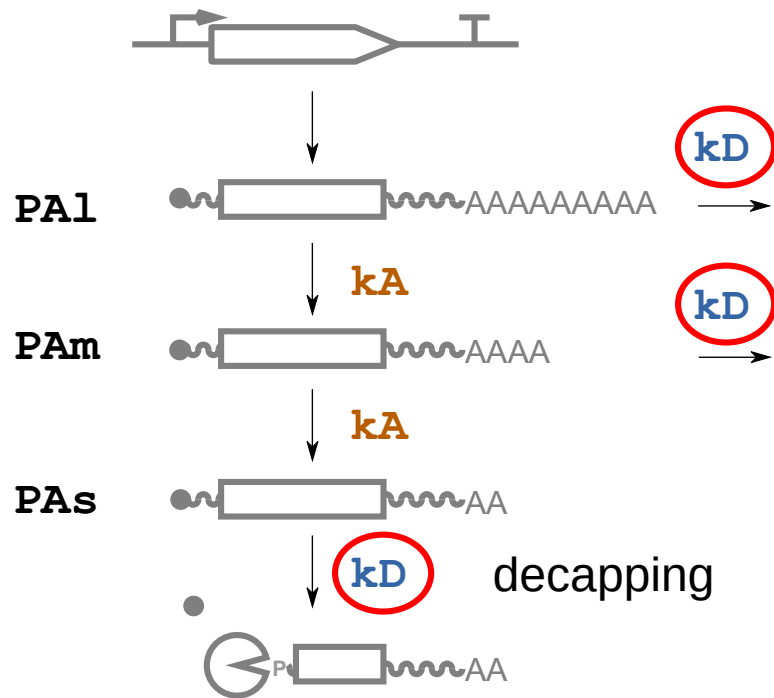
“After their tails become short, mRNAs decay at rates that span a **1000-fold** range”

Cao et al., *RNA* 2001



MFA2pG decapping rate is **17x** faster than that of *PGK1pG*

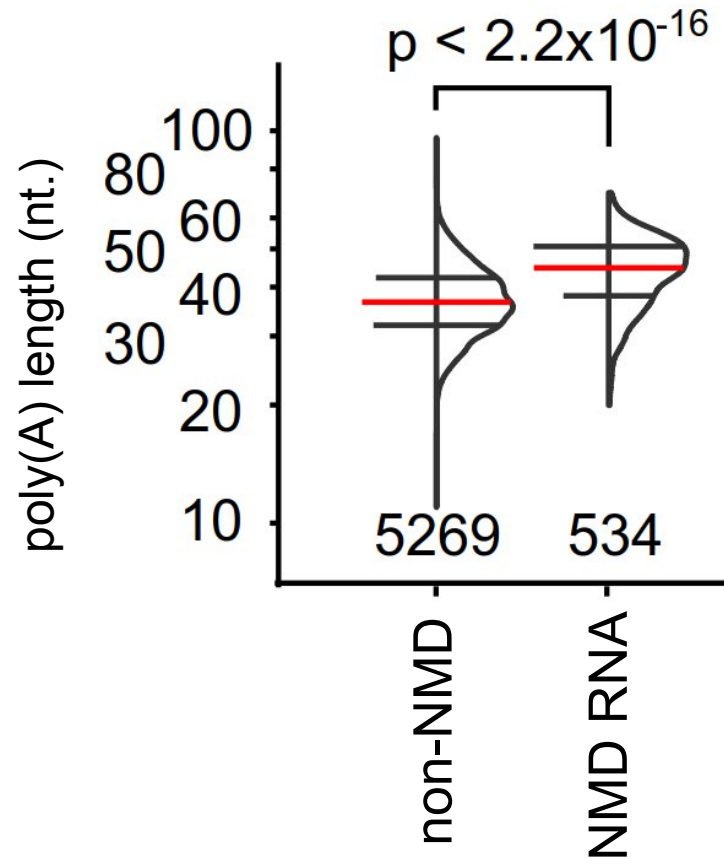
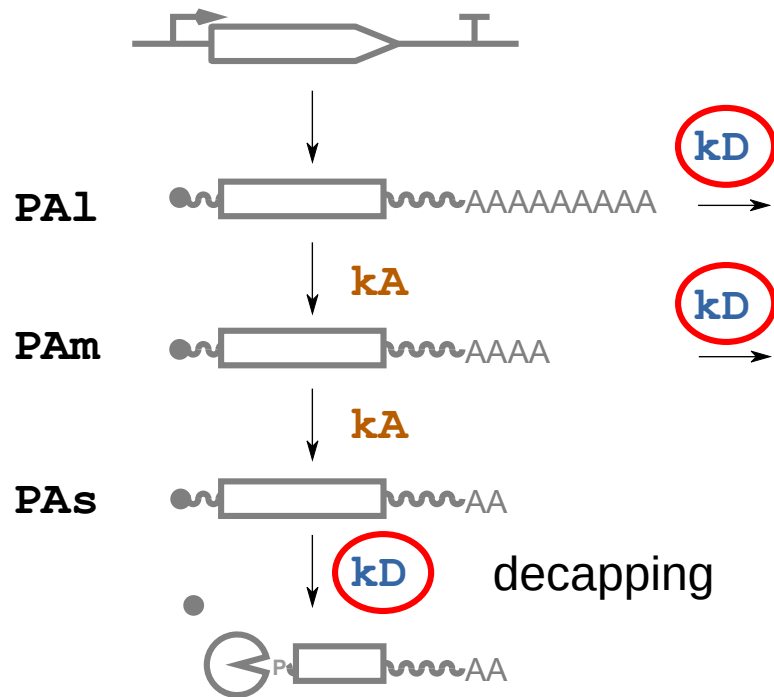
Alternative hypothesis: decapping **without** prior deadenylation



Swisher & Parker, *PLoS One* 2011
He & Jacobson, *RNA* 2014
Dehecq et al., *EMBOJ* 2018

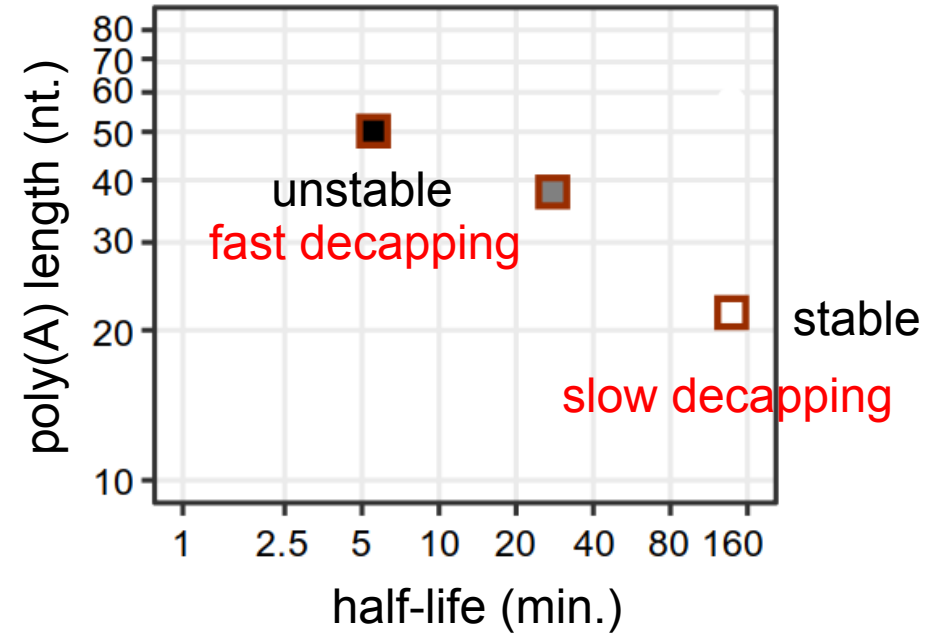
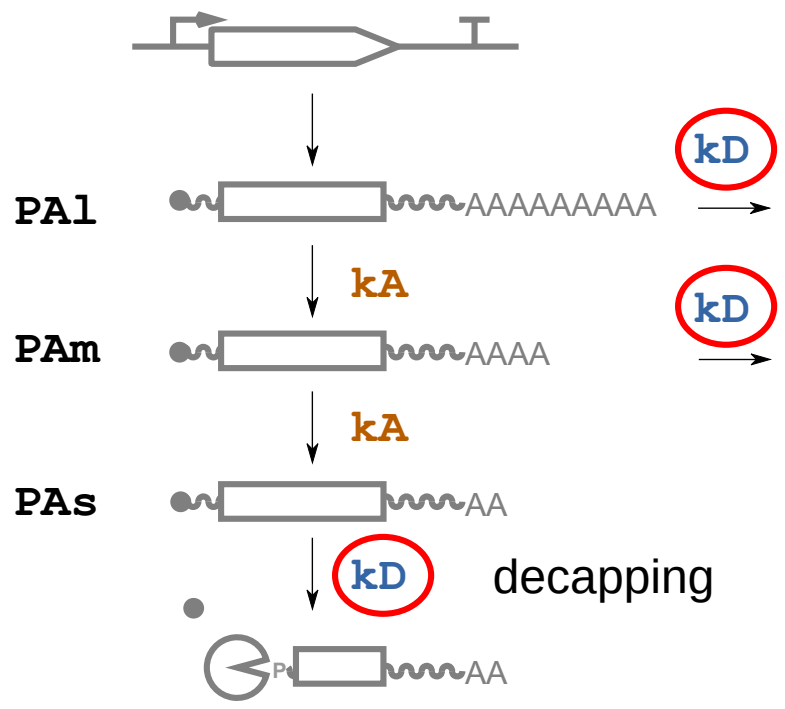
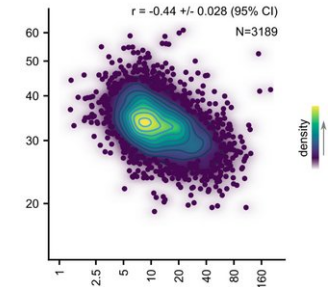
Muhlrad & Parker, *Nature* 1994

NMD substrates (unstable) have longer than average poly(A) tails

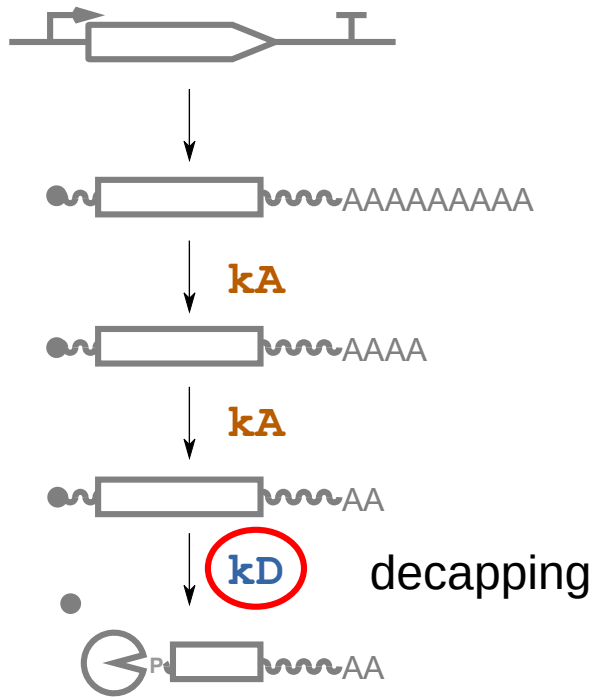


data from Tudek et al., *Nat Commun* 2021

Decapping without prior deadenylation can explain steady-state results

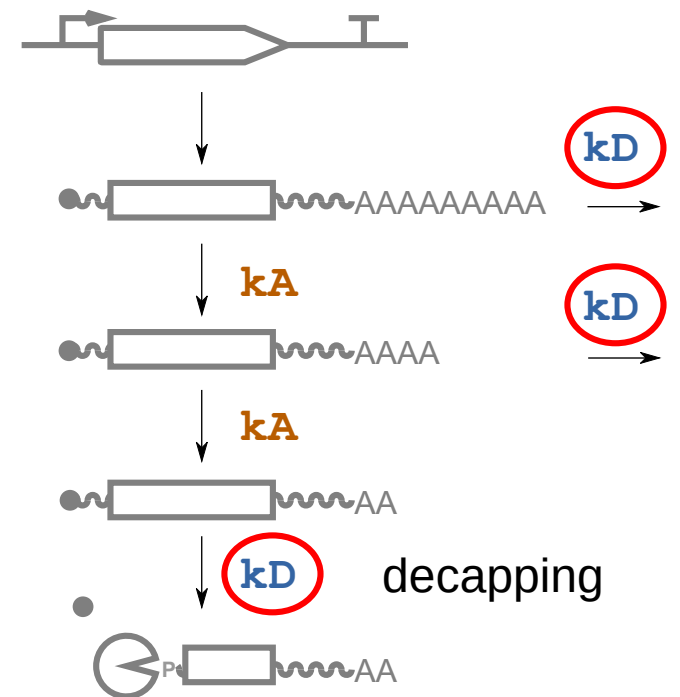


Large scale results explained by a **highly variable decapping rate** in both models

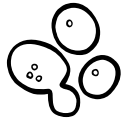


with prior deadenylation

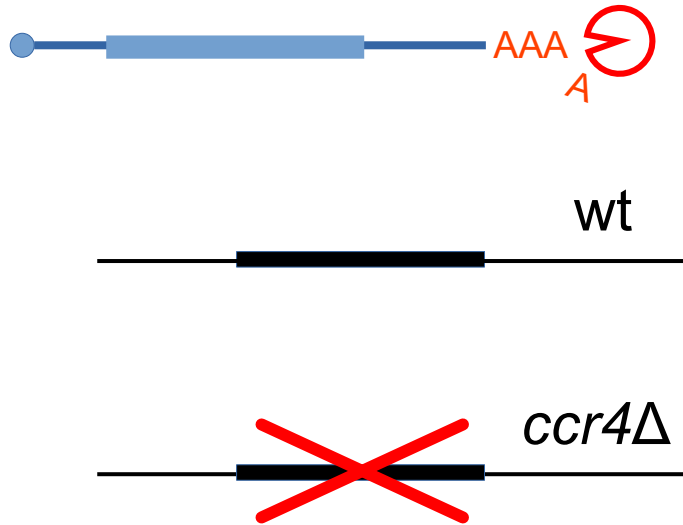
?



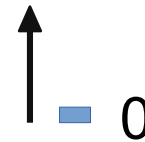
without prior deadenylation
NMD and similar to NMD



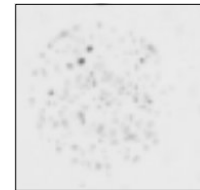
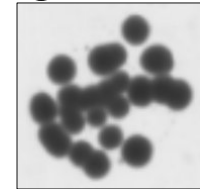
Deadenylation mutants grow slowly



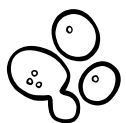
deadenylase levels



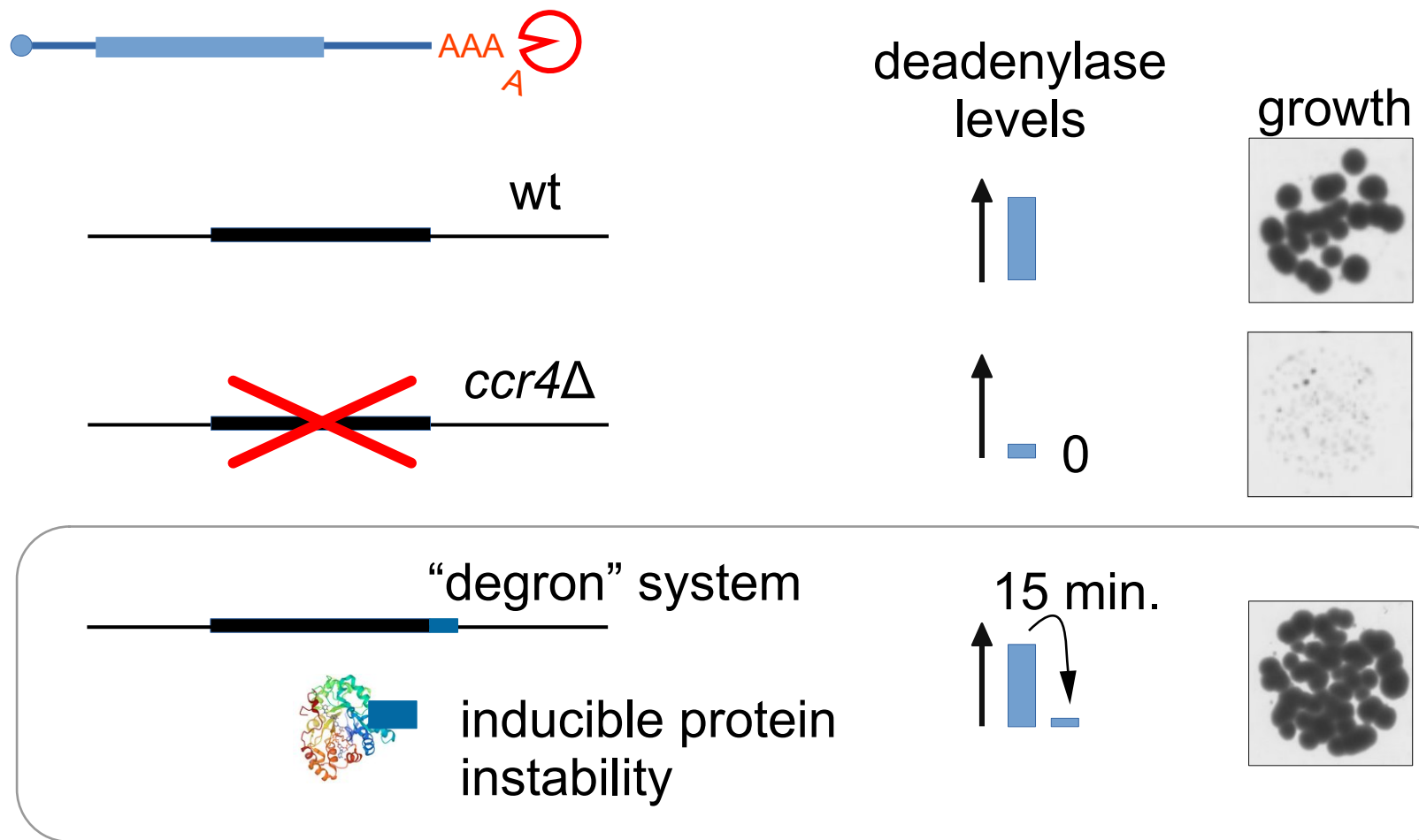
growth

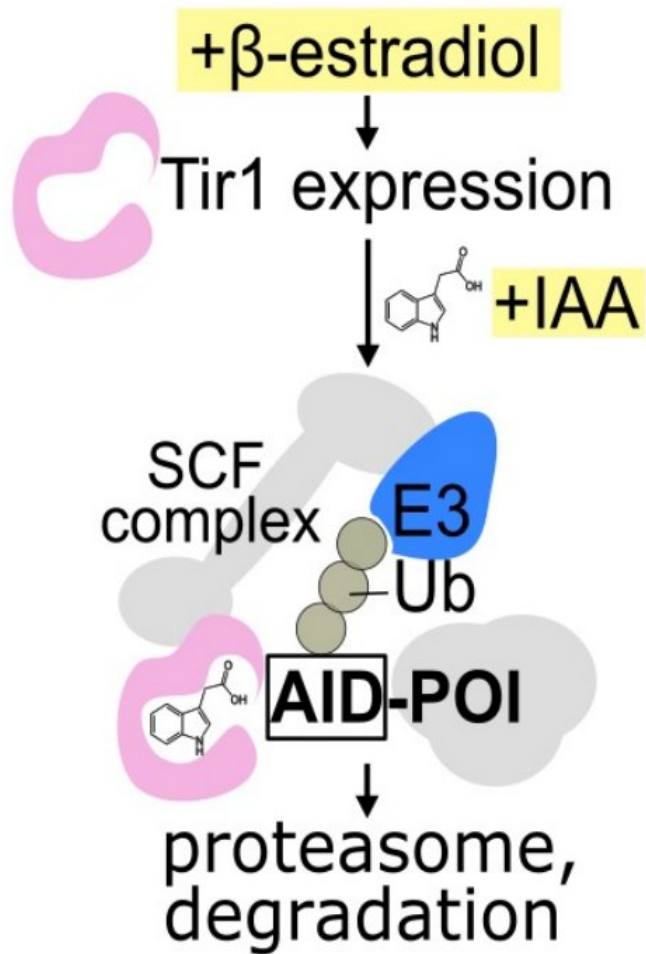


What to do ?

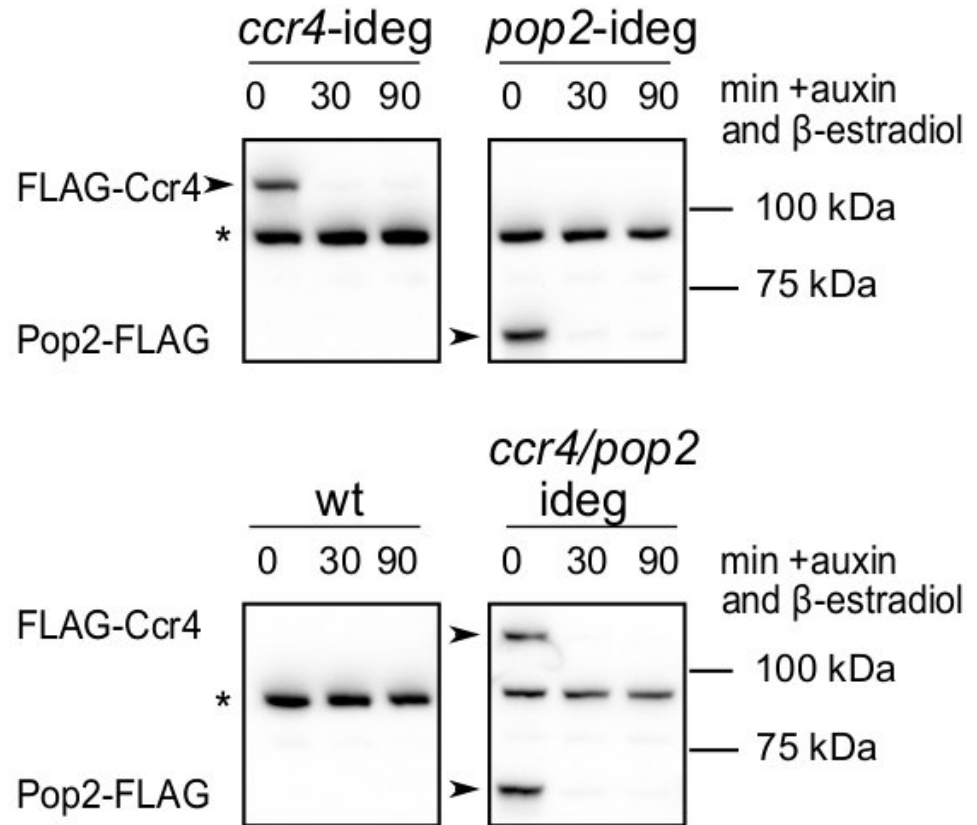


A degron tool to test mRNA deadenylation effects

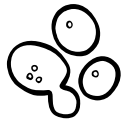




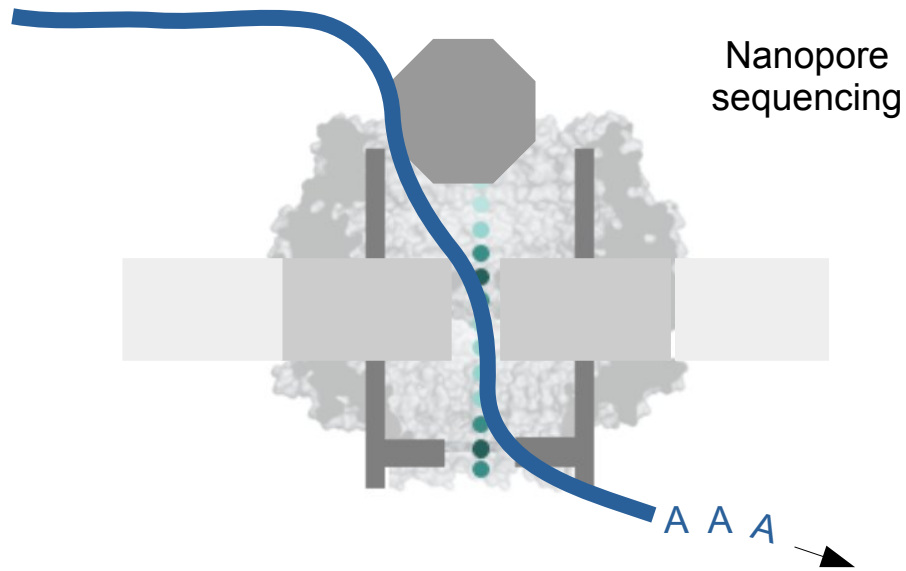
An inducible degron system



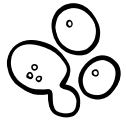
Problem: How to estimate the effects of deadenylation enzyme depletion ?



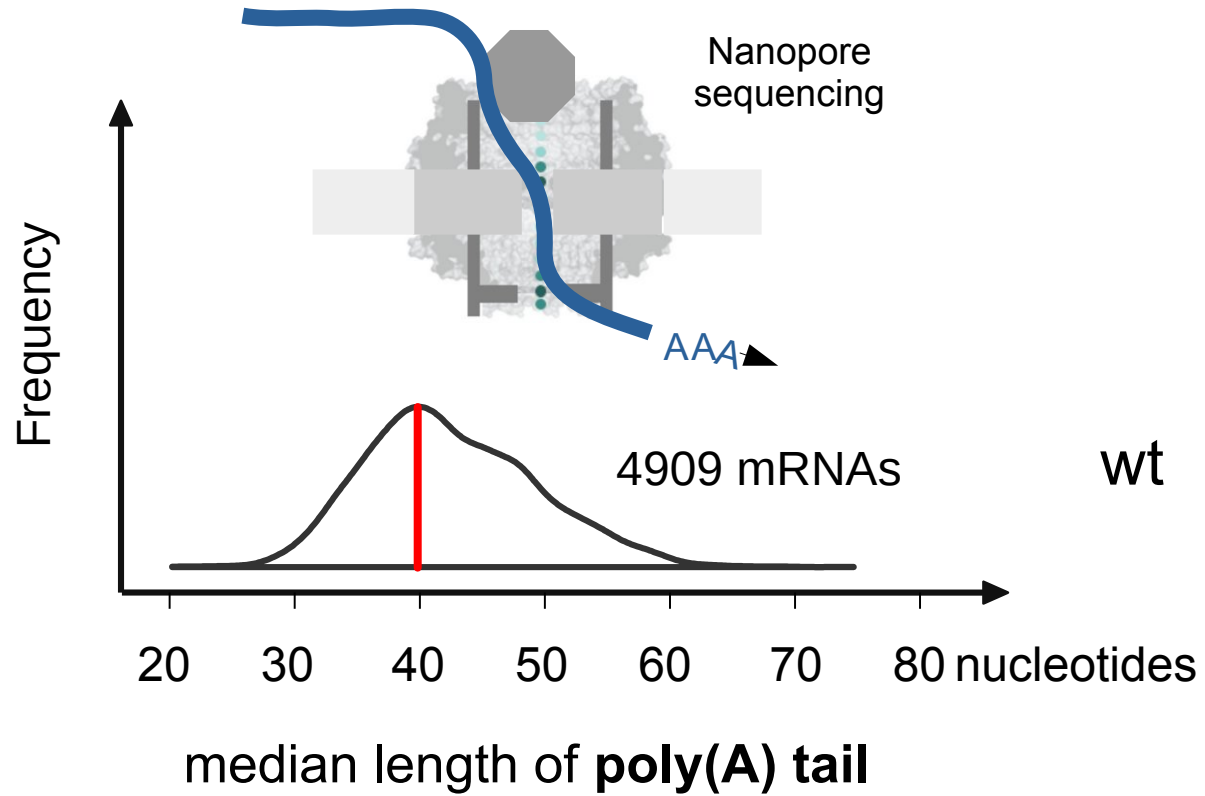
Nanopore sequencing estimates of poly(A)

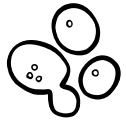


Workman et al., *Nature Methods* 2019
Tudek et al., *Nature Commun* 2021

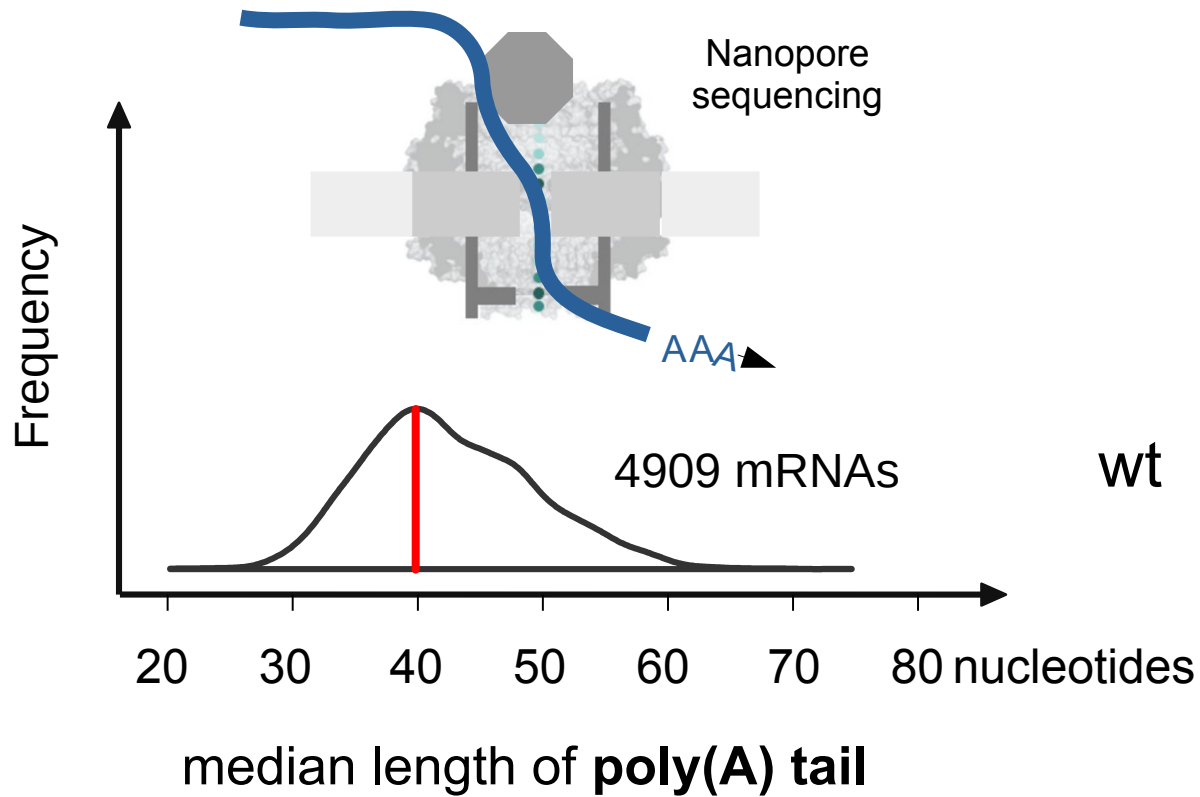


Nanopore sequencing estimates of poly(A)

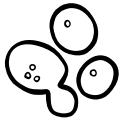




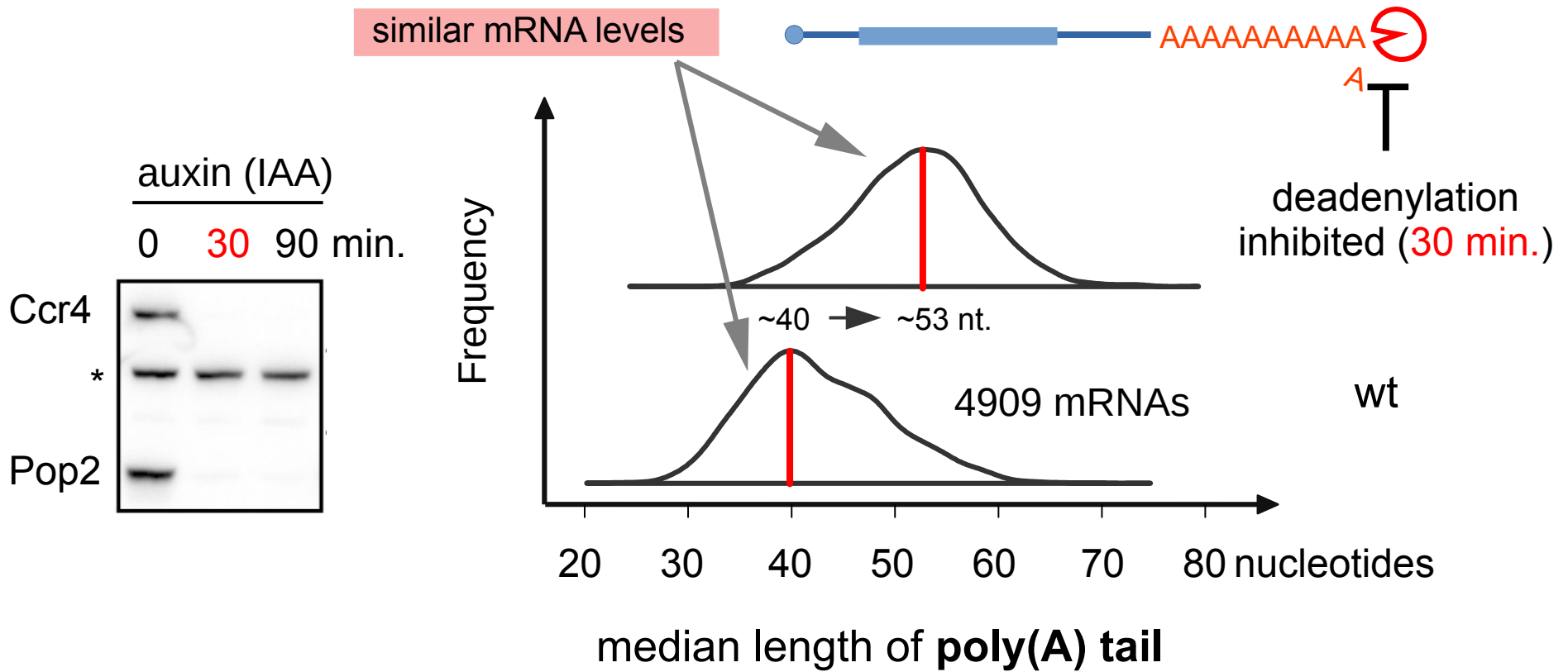
Nanopore sequencing estimates of poly(A)

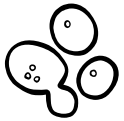


Problem: What do you expect in a strain where deadenylation is inhibited ?

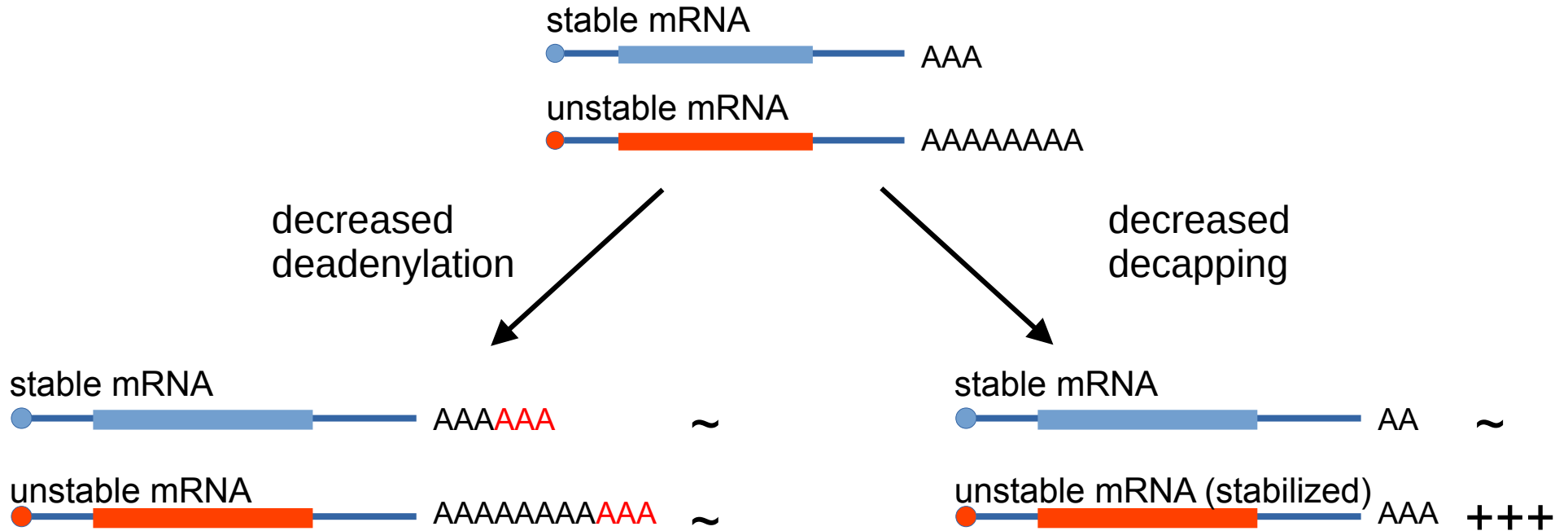


No correlated mRNA levels changes after deadenylases depletion

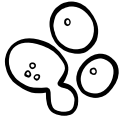




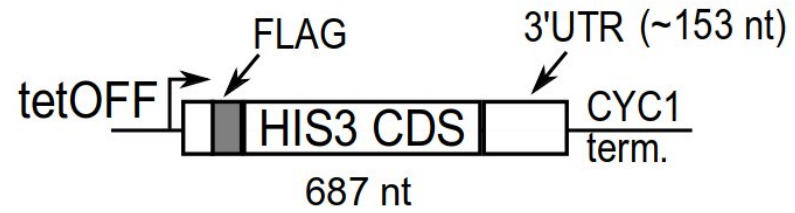
Interpretation: poly(A) length is a witness of mRNA **stability**



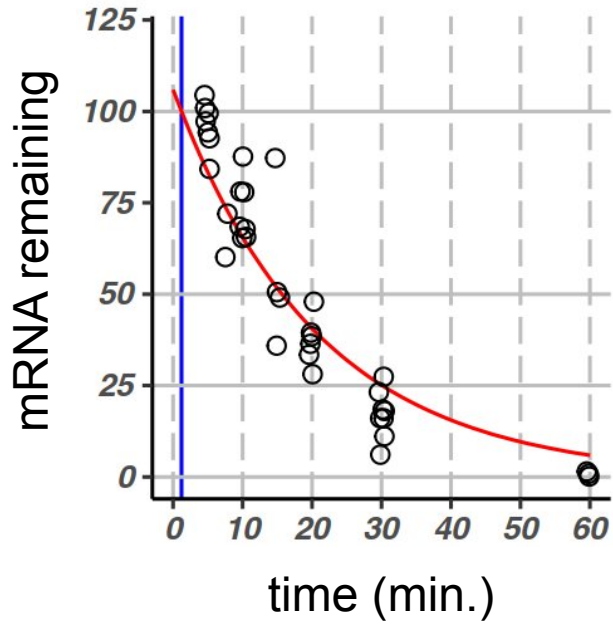
Compatible with **deadenylation-independent** mRNA degradation
Incompatible with a deadenylation-dependent model



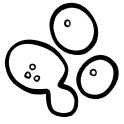
Reporter mRNA stability



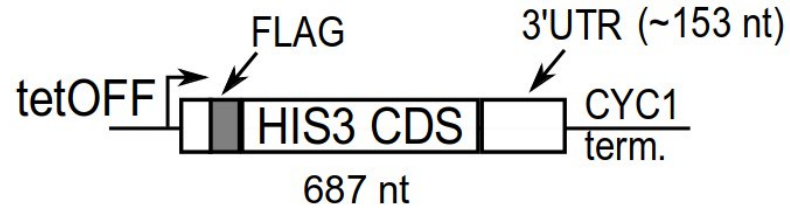
half-life 15.7 +/- 1.7 min.



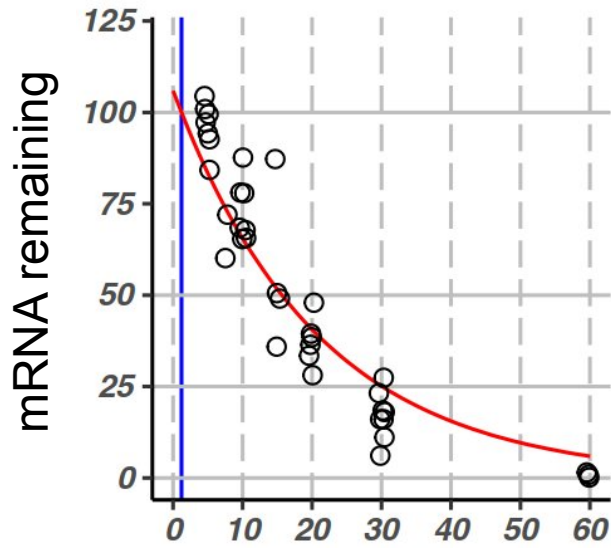
wt



Decapping is important for reporter degradation



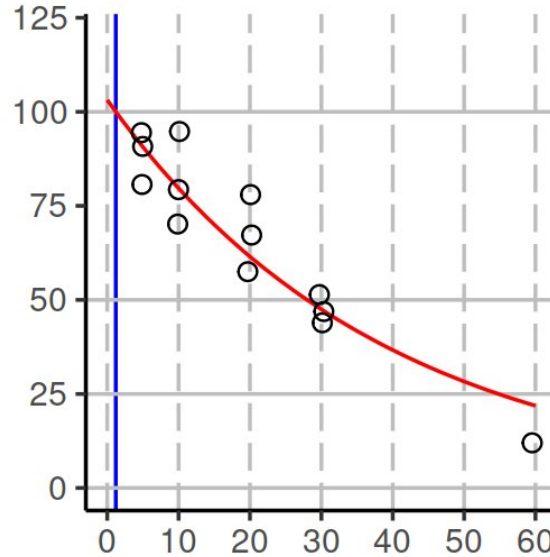
half-life 15.7 +/- 1.7 min.



time (min.)

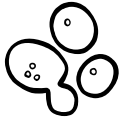
wt

28 +/- 4.8 min.

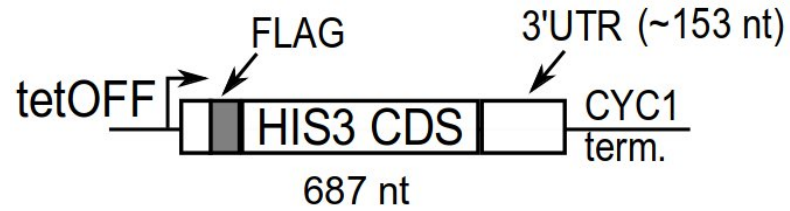


time (min.)

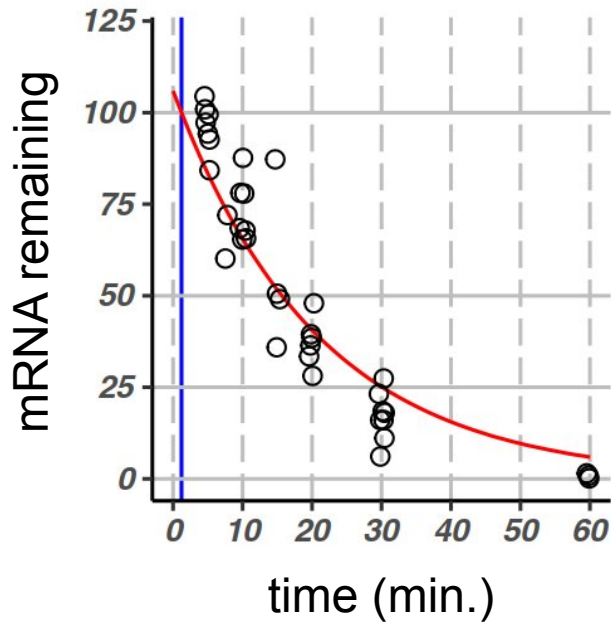
Dcp2 depl.



No change in reporter stability when slowing down deadenylation

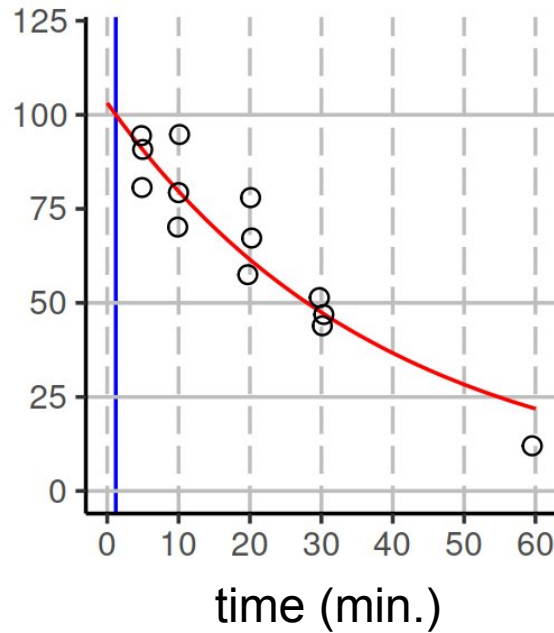


half-life 15.7 +/- 1.7 min.



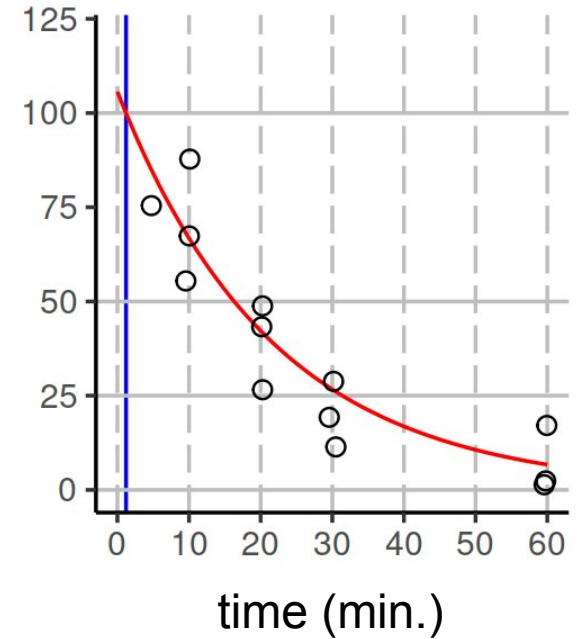
wt

28 +/- 4.8 min.

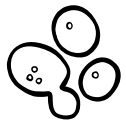


Dcp2 depl.

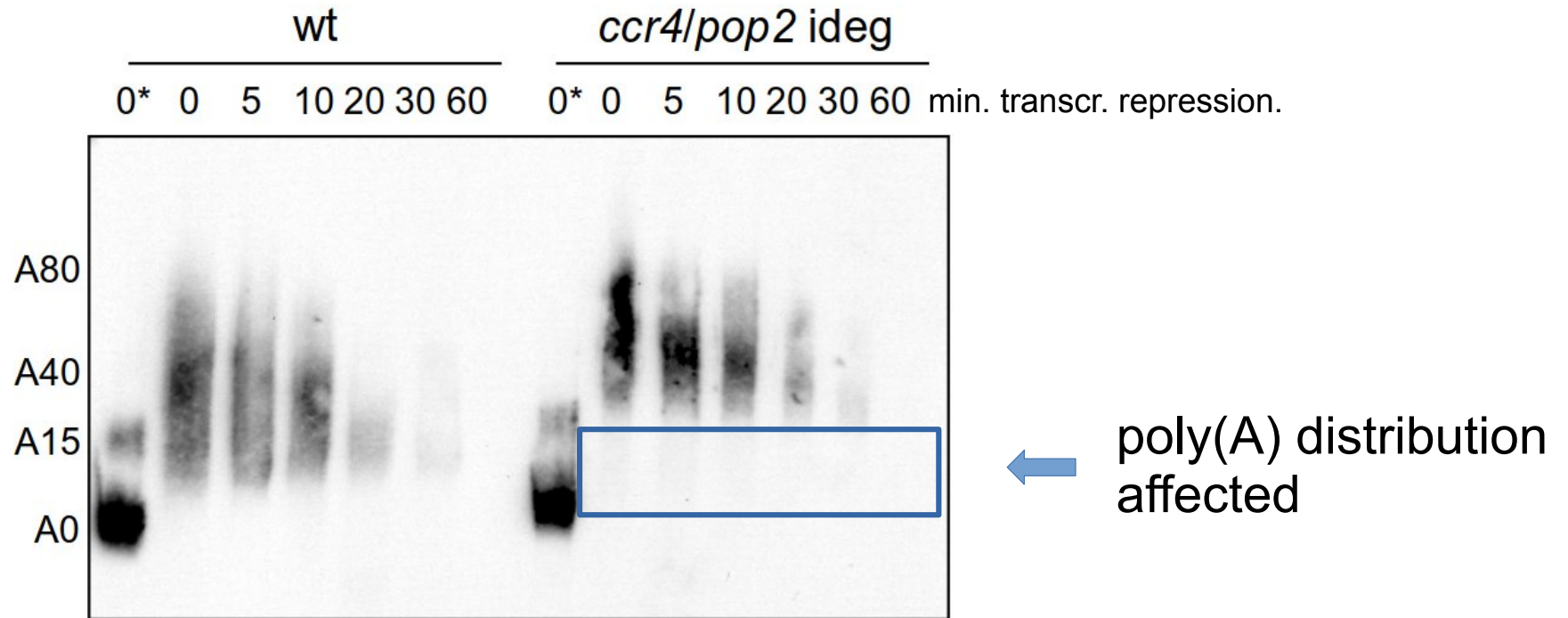
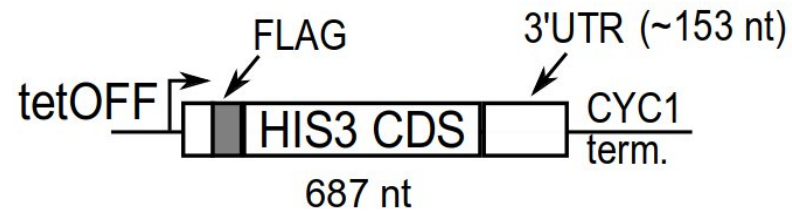
16.3 +/- 5.3 min.

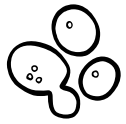


Ccr4/Pop2 depl.

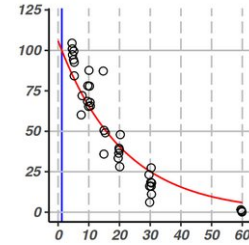
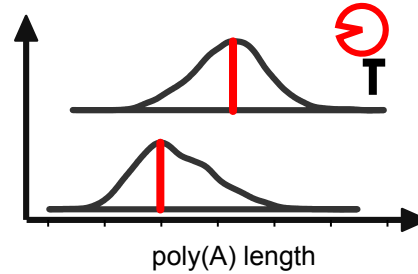
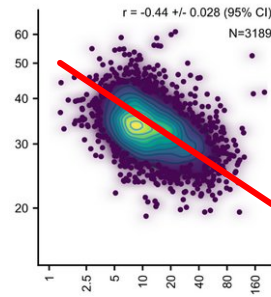
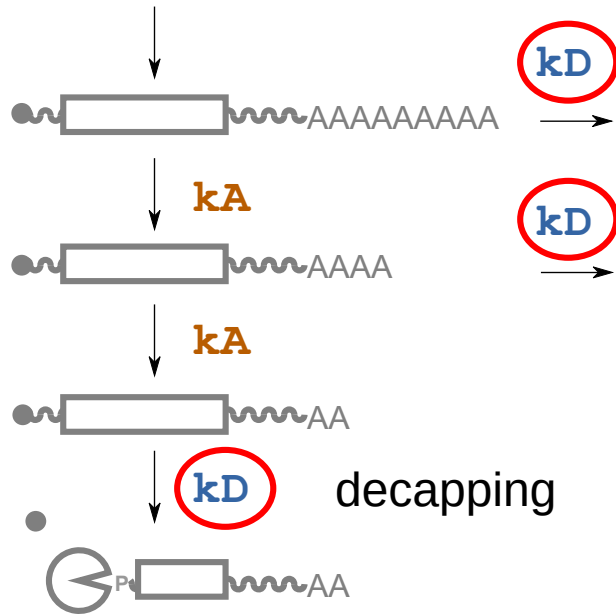


No change in reporter stability when slowing down deadenylation



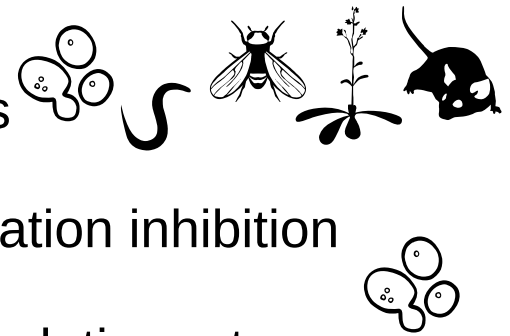


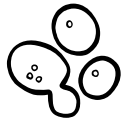
Conclusions



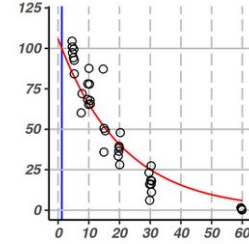
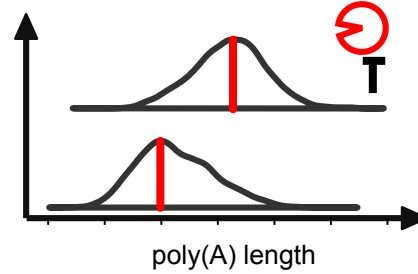
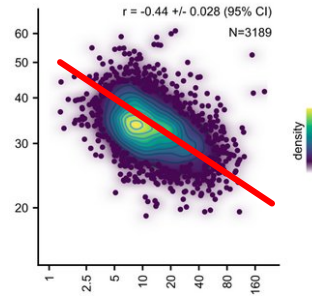
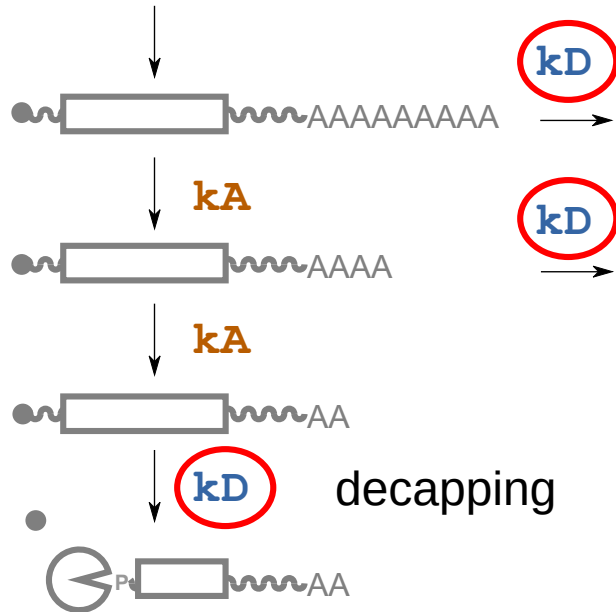
Deadenylation-independent RNA degradation explains:

- long poly(A) of unstable mRNAs
- lack of global effect of deadenylation inhibition
- reporter mRNA unaffected degradation rate





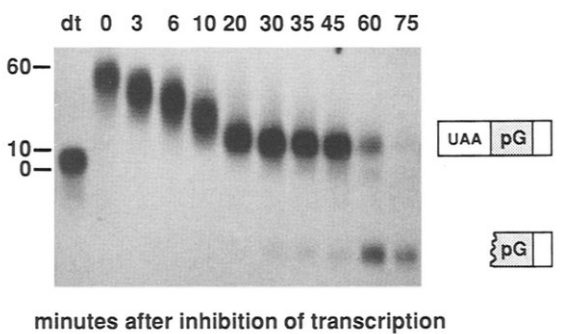
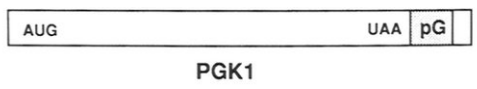
Implications



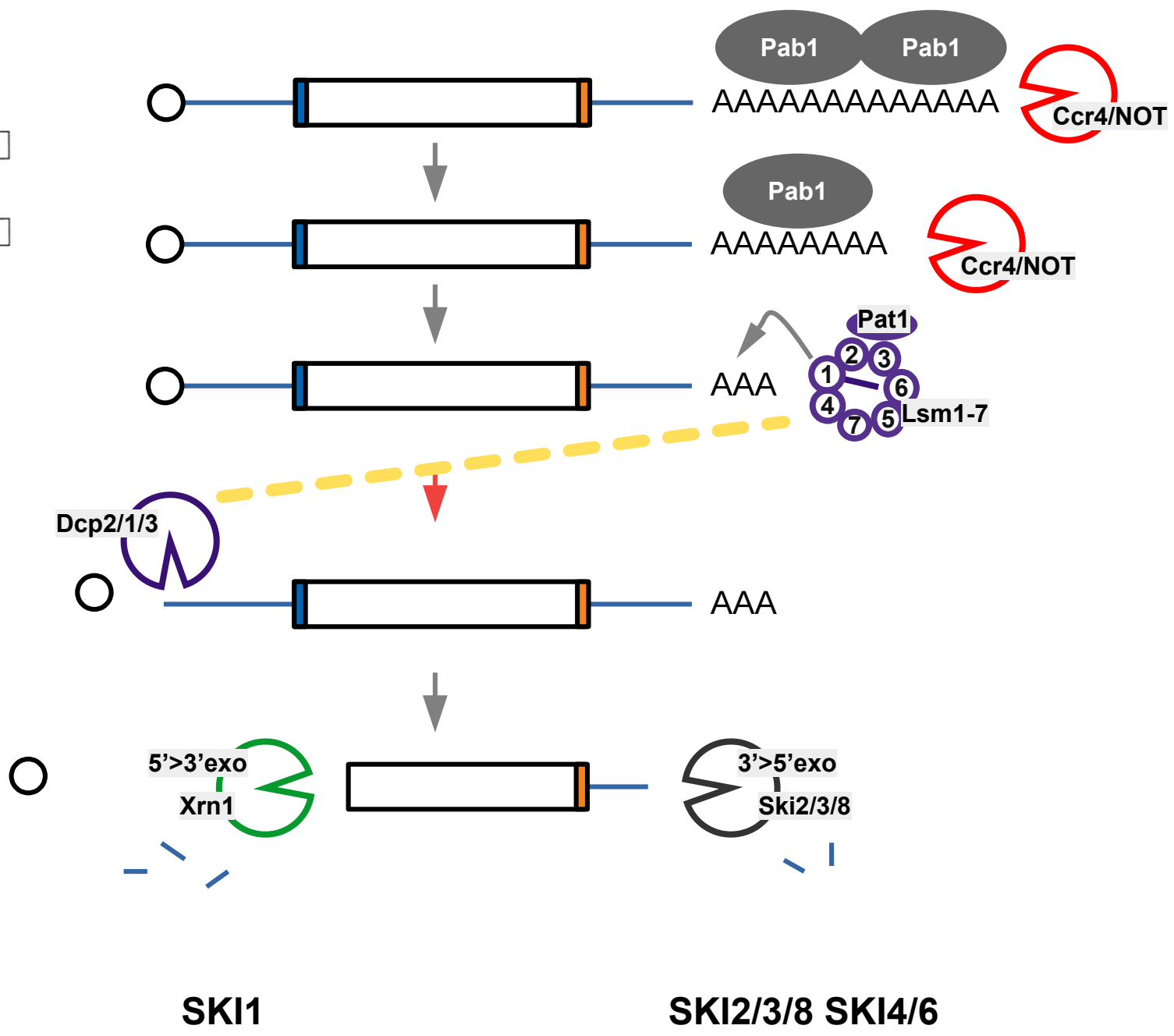
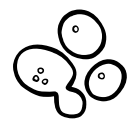
We can:

- estimate deadenylation and decapping rates from steady-state poly(A) length results
- re-analyse previous results

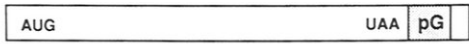
Textbook RNA degradation schematics



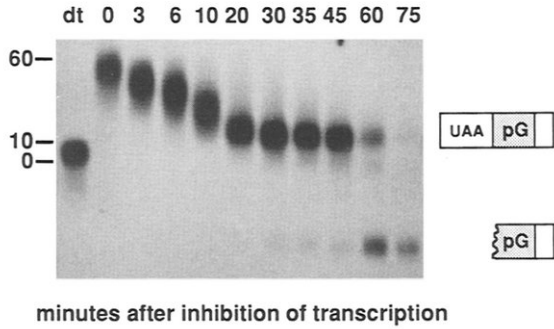
Decker & Parker, *Genes & Dev* 1993



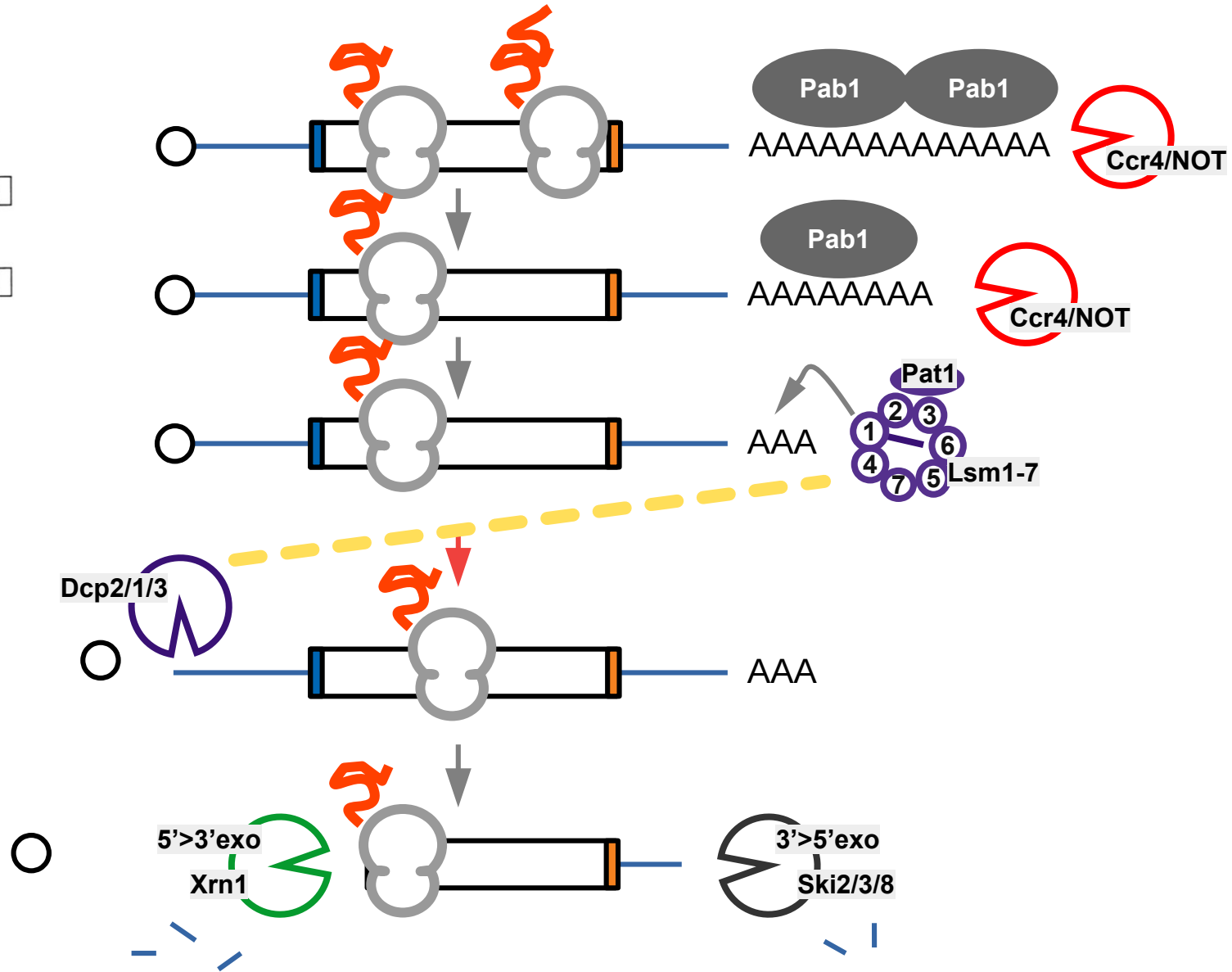
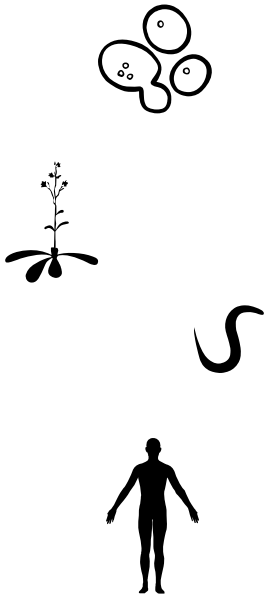
Ribosomes are essential for mRNA stability!



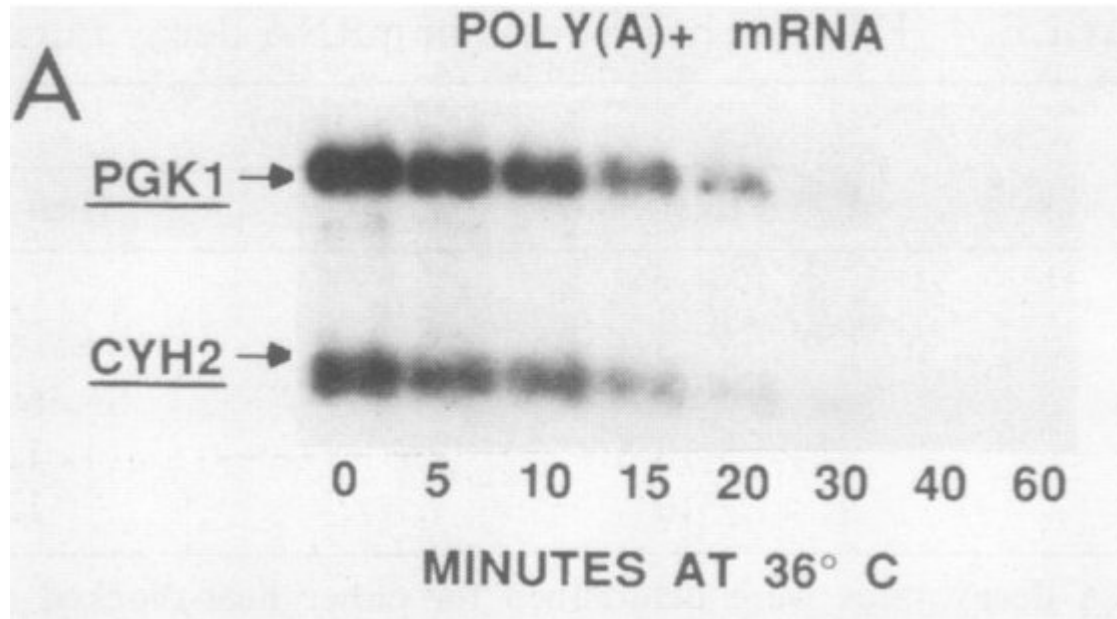
PGK1



Decker & Parker,
Genes & Dev 1993

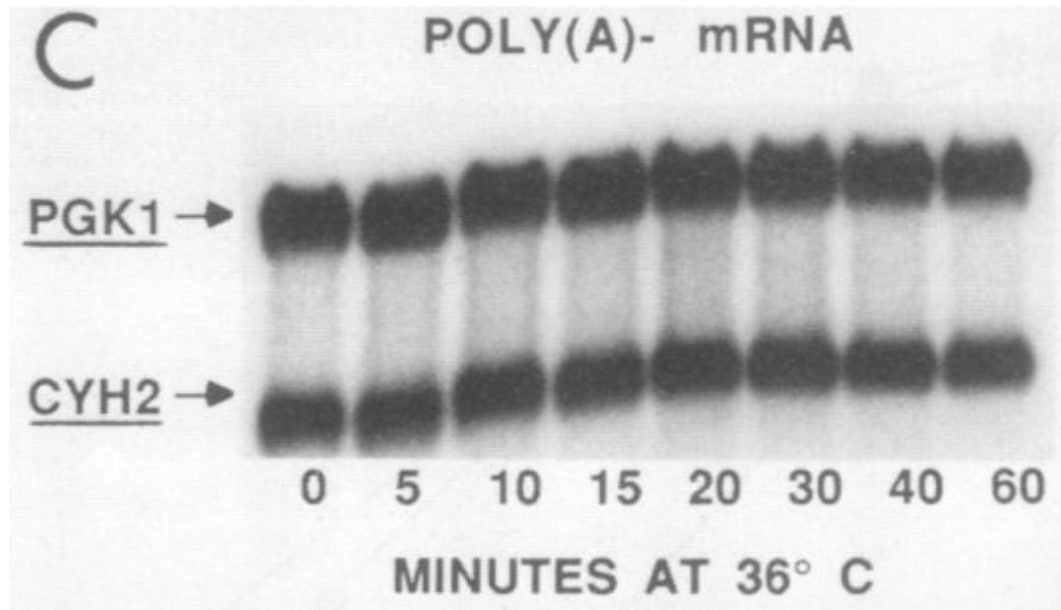


Deadenylation kinetics and mRNA degradation (published results reinterpretation)



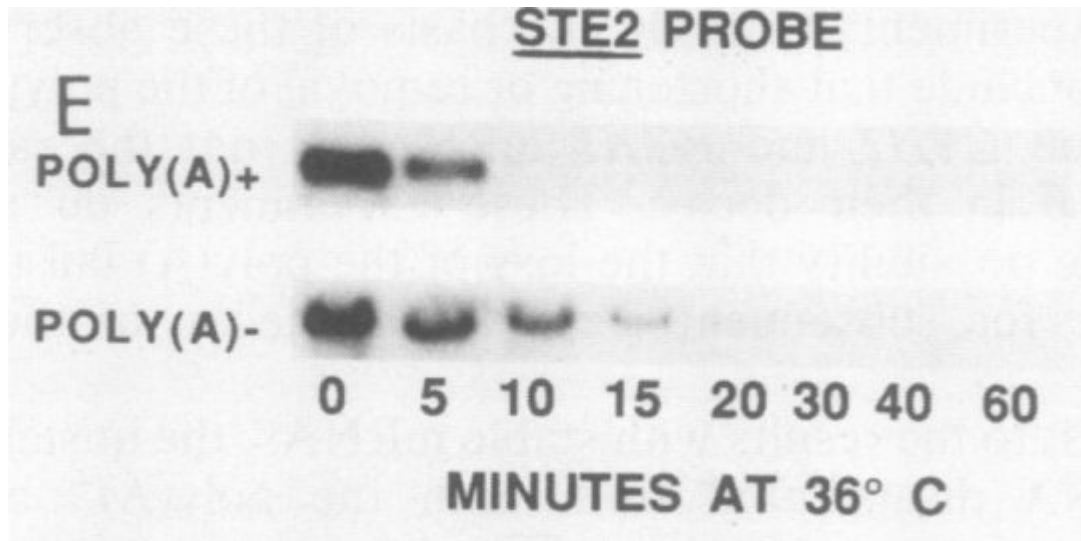
Reinterpretation:

Deadenylation (?)
half-life of 15 mins.



Degradation
half-life of >60 mins.

Deadenylation kinetics and mRNA degradation (published results reinterpreted)



Reinterpretation:

Deadenylation+degradation
half-life of 5 mins.

Degradation
half-life of 5-10 mins.