ncRNAs in yeast introns

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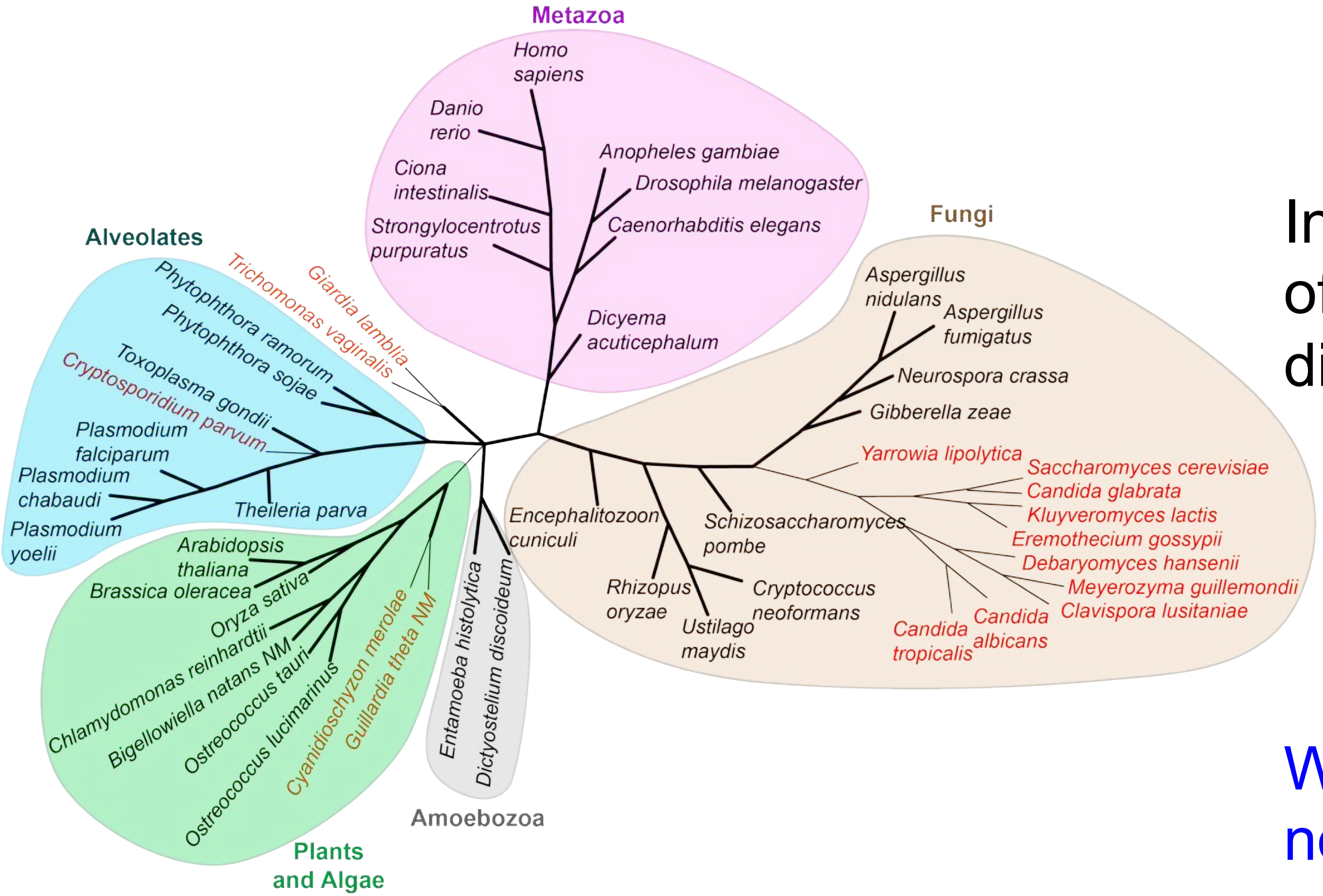
Introns and evolution

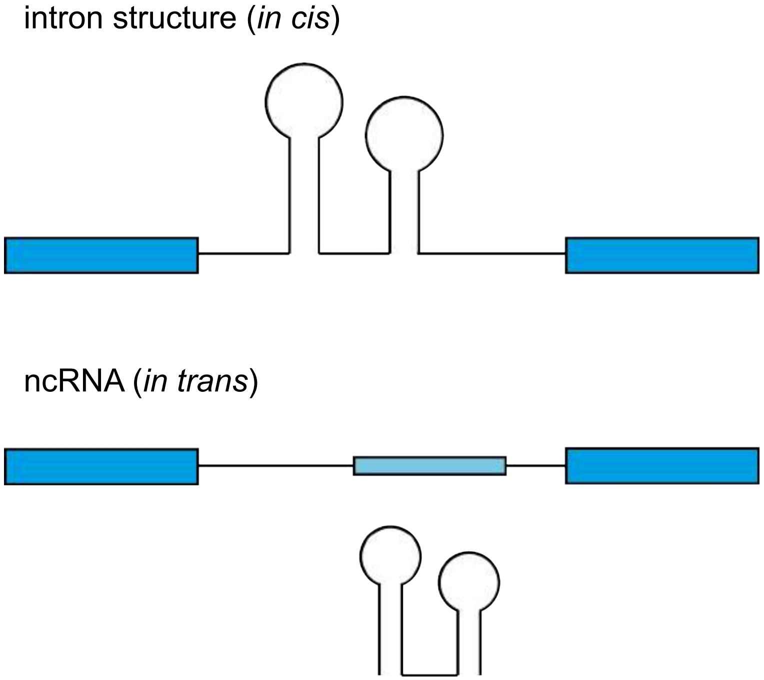


Adapted from Irimia *et al.* (2008)

Independent loss of introns in different branches

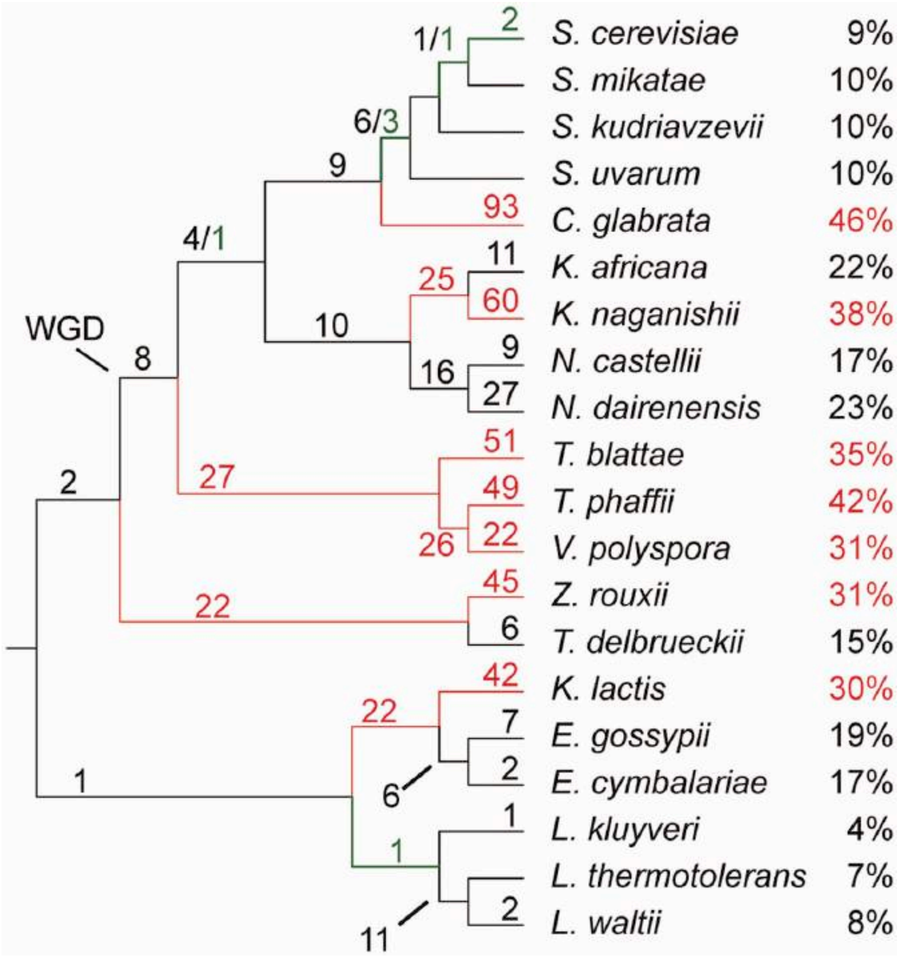
Why loss is not complete?

Reasons for intron retention



- Evolutionary remains
- Functional:
  - Splicing
  - Sequence of intron
  - Structure of intron
  - Non-coding RNA genes

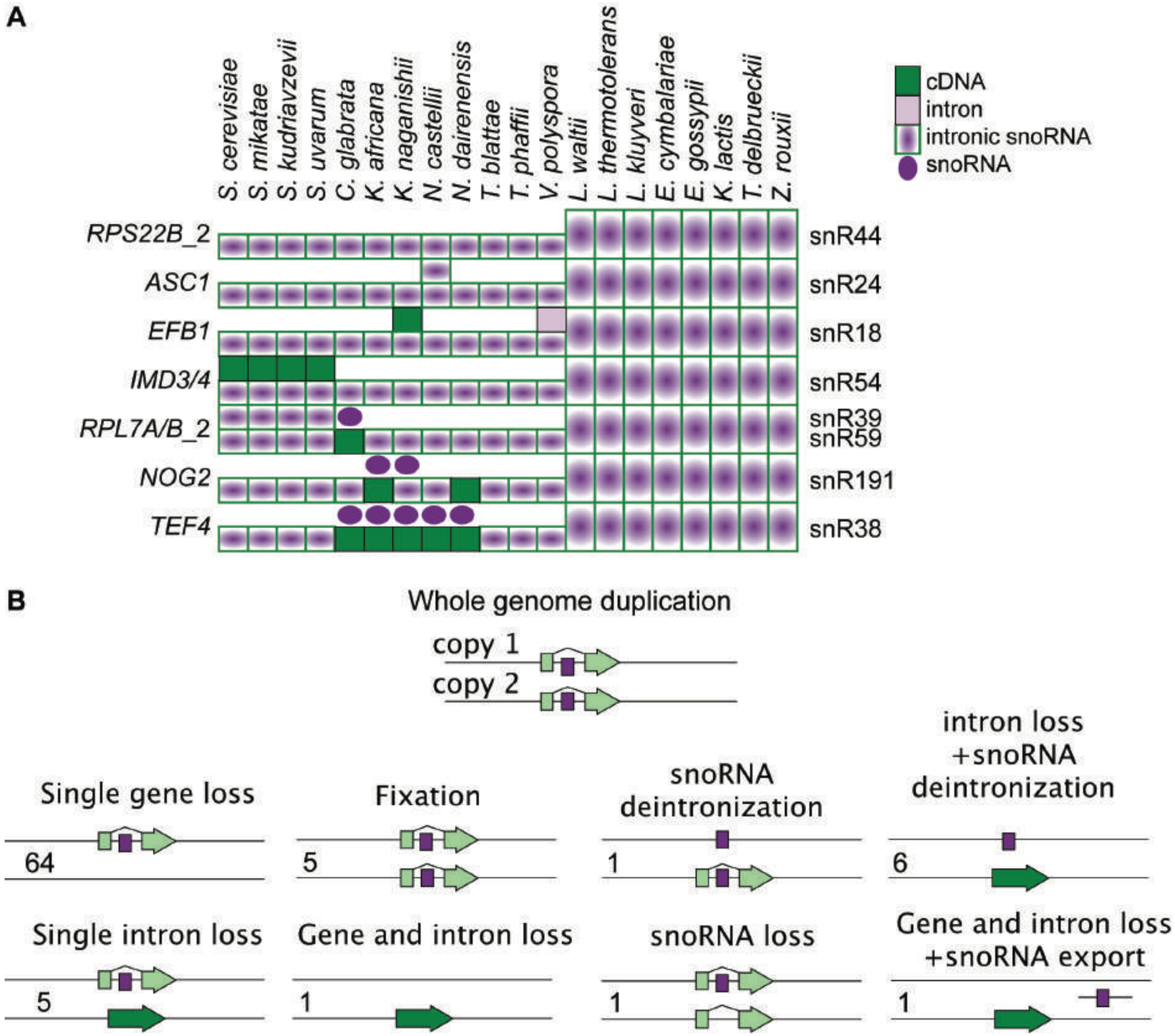
Intron evolution in Saccharomycotina



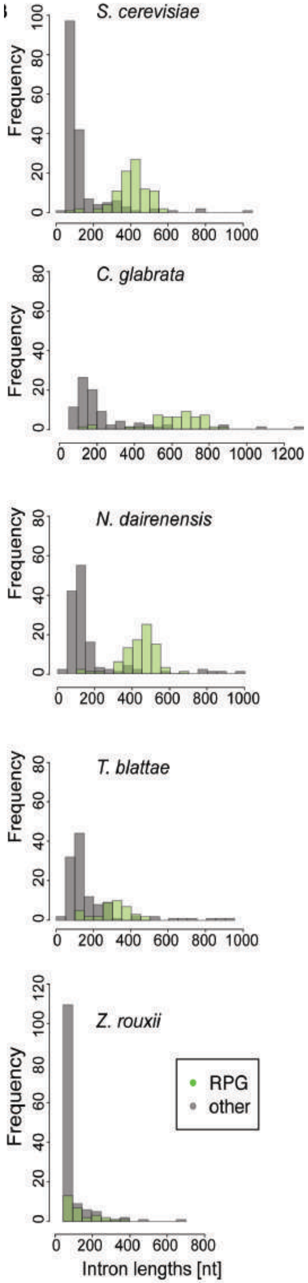
Hooks *et al.*, Genome biology and evolution (2014)

- WGD
- 250 orthologous introns in 20 species
- Perfect intron losses (gene replaced with its mRNA by homologous recombination)
- Some evidence for micro-homology mediated loss

Intronic ncRNA (*in trans*)

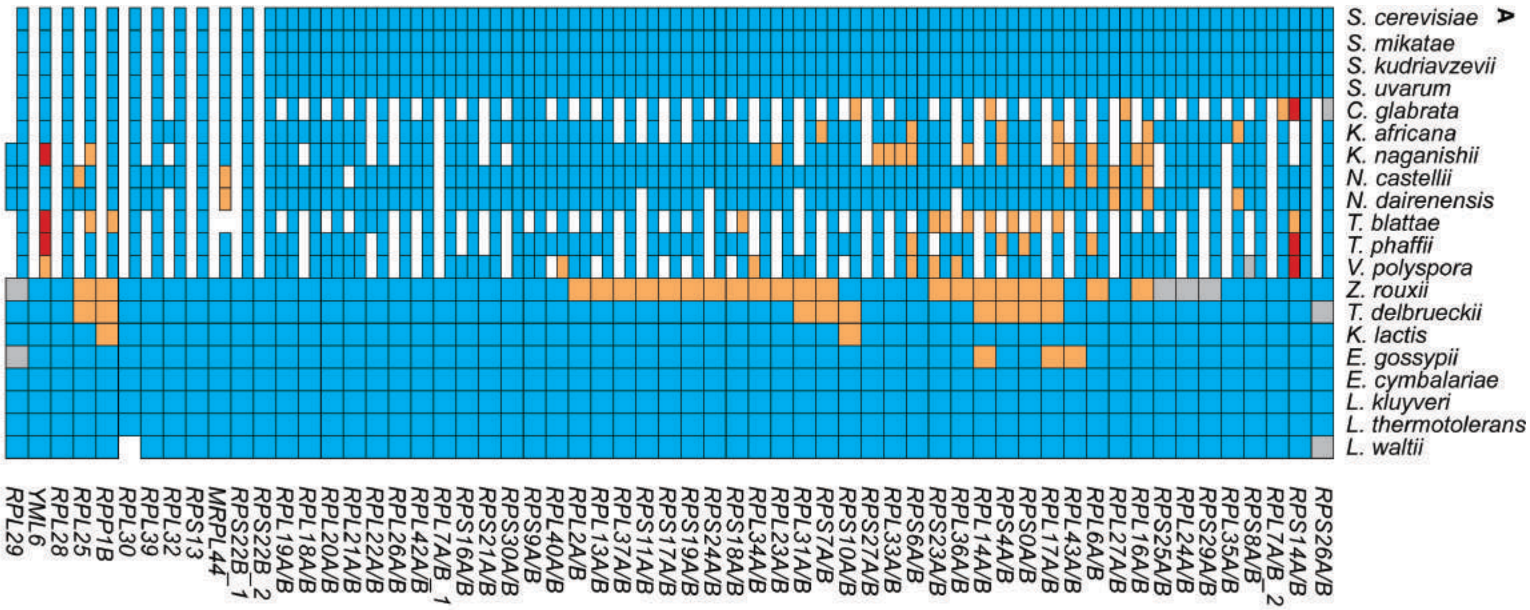


Hooks *et al.*, Genome biology and evolution (2014)

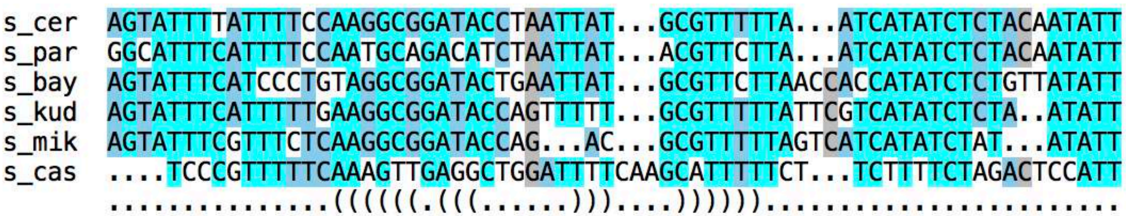


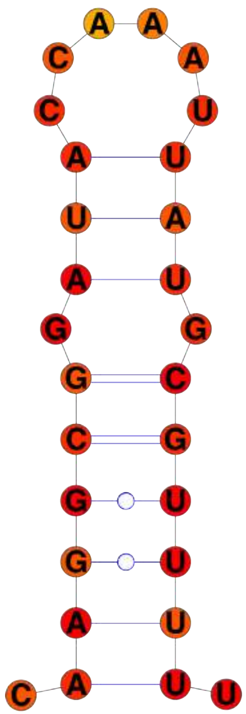
Intron structure (*in cis*)

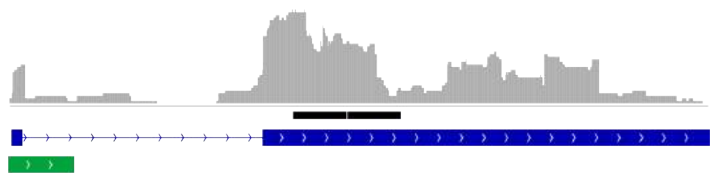
Hooks *et al.*, Genome biology and evolution (2014)

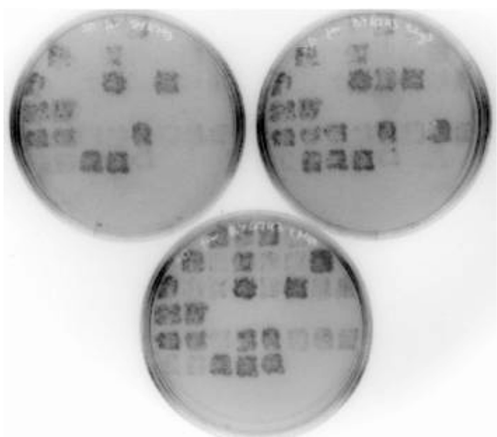


Approach









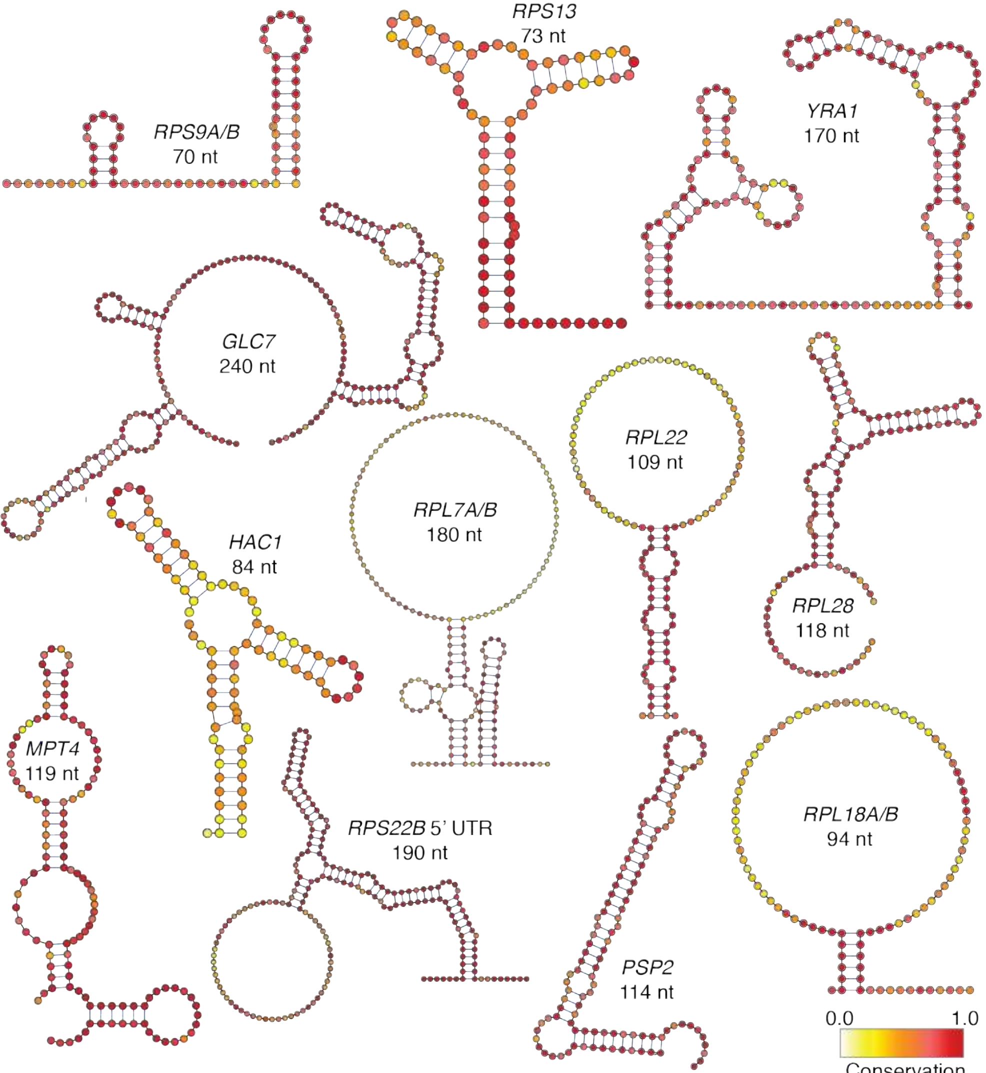
306 introns

- orthologs (genes)
- alignments (*mafft*)
- ncRNA prediction (*RNAz*, *cmfinder*, *Evofold* + *infernal*)

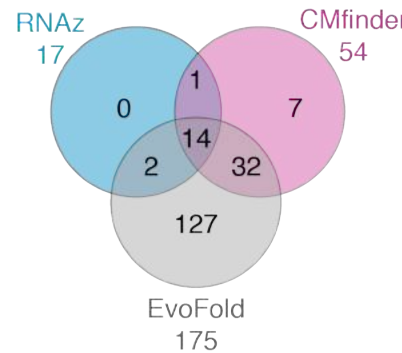
~20 candidates

- RT-PCR
- Northern
- RNAseq
- knock-outs

Computational screen



Hooks *et al.*, Genetics (2016)

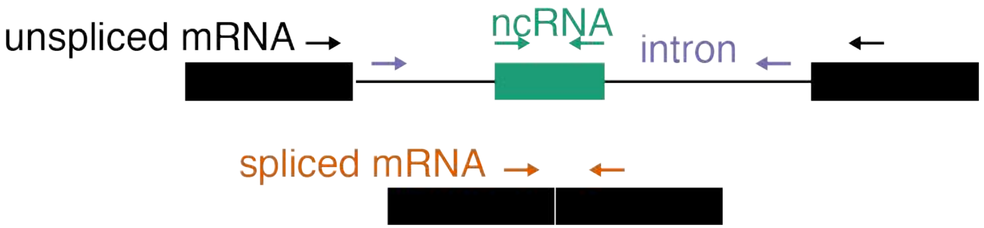


- 14 intersecting predictions
+ 2 paralogs
+ 3 other high-scoring

- conserved RNA structures up to *Candida* sp.

- only two from 19 RNA structures predicted in introns have not been shown to have function or a phenotype when deleted

RT-PCRs

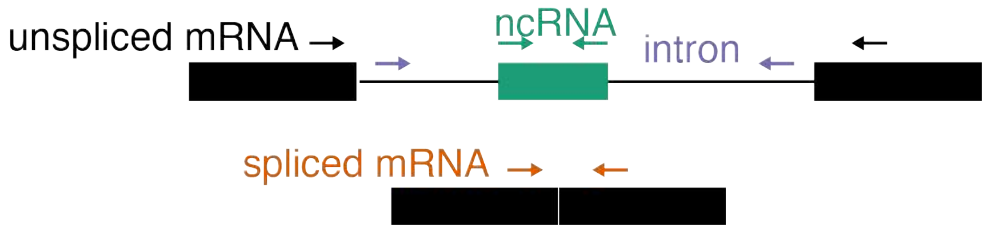


	intronic ncRNA
mRNA	spliced
intron	-
ncRNA	++

snoRNAs
putative ncRNA

Hooks *et al.*, Genetics (2016)

RT-PCRs

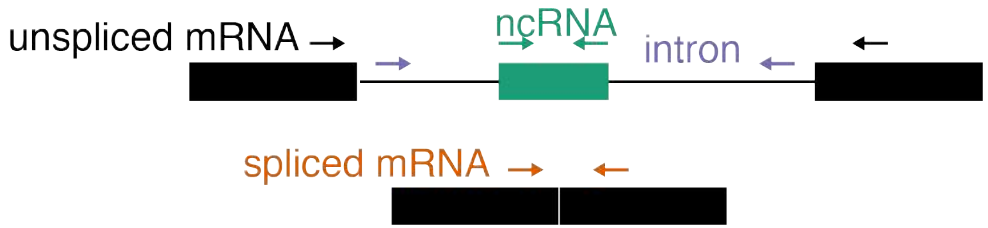


	intronic ncRNA	intron maintained	intron retained
mRNA	spliced	spliced	spliced/unspliced
intron	-	+	+
ncRNA	++	++	++

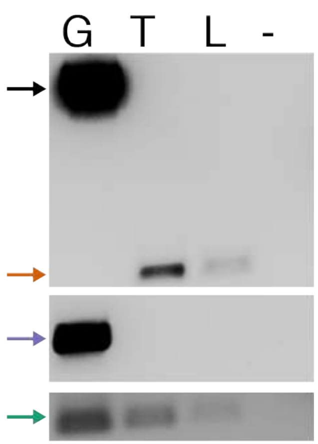
snoRNAs
putative ncRNA

Hooks *et al.*, Genetics (2016)

RT-PCRs

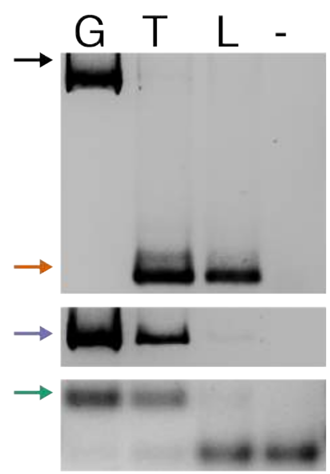


intronic ncRNA



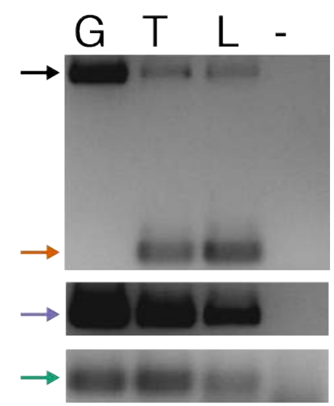
RPS22B (snR44)
GLC7
RPL7B
MTP5

intron maintained



NOG2 (snR191)
RPS13
RPS9B
RPL7A
RPL22A
RPS22B 5'UTR

intron retained



IMD4 (snR54)
PSP2
RPS9A
RPL28
RPL22B
RPL18A/B
YRA1

Hooks *et al.*, Genetics (2016)

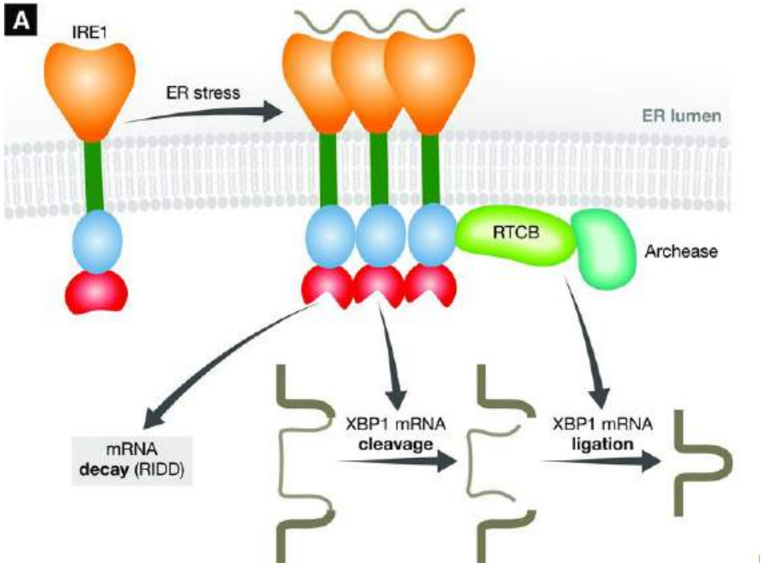
RNA structure and introns

1. *HAC1 / XBP1*

2. *GLC7*

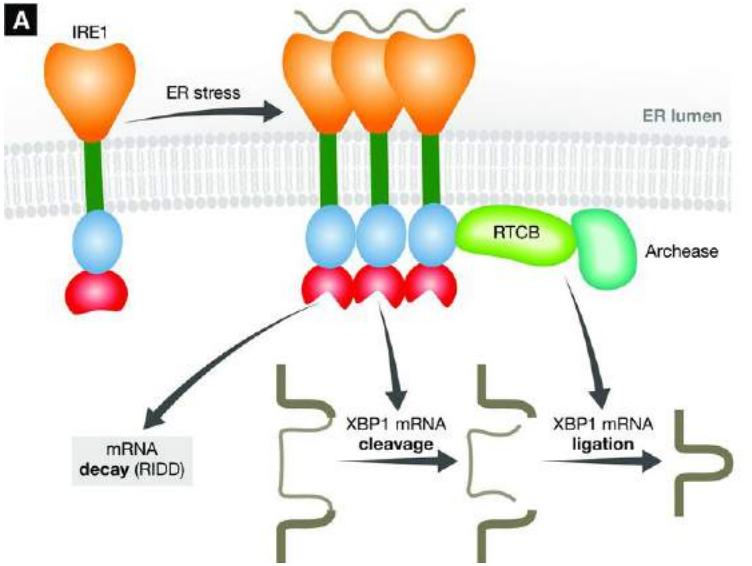
3. *RPL28*

HAC1

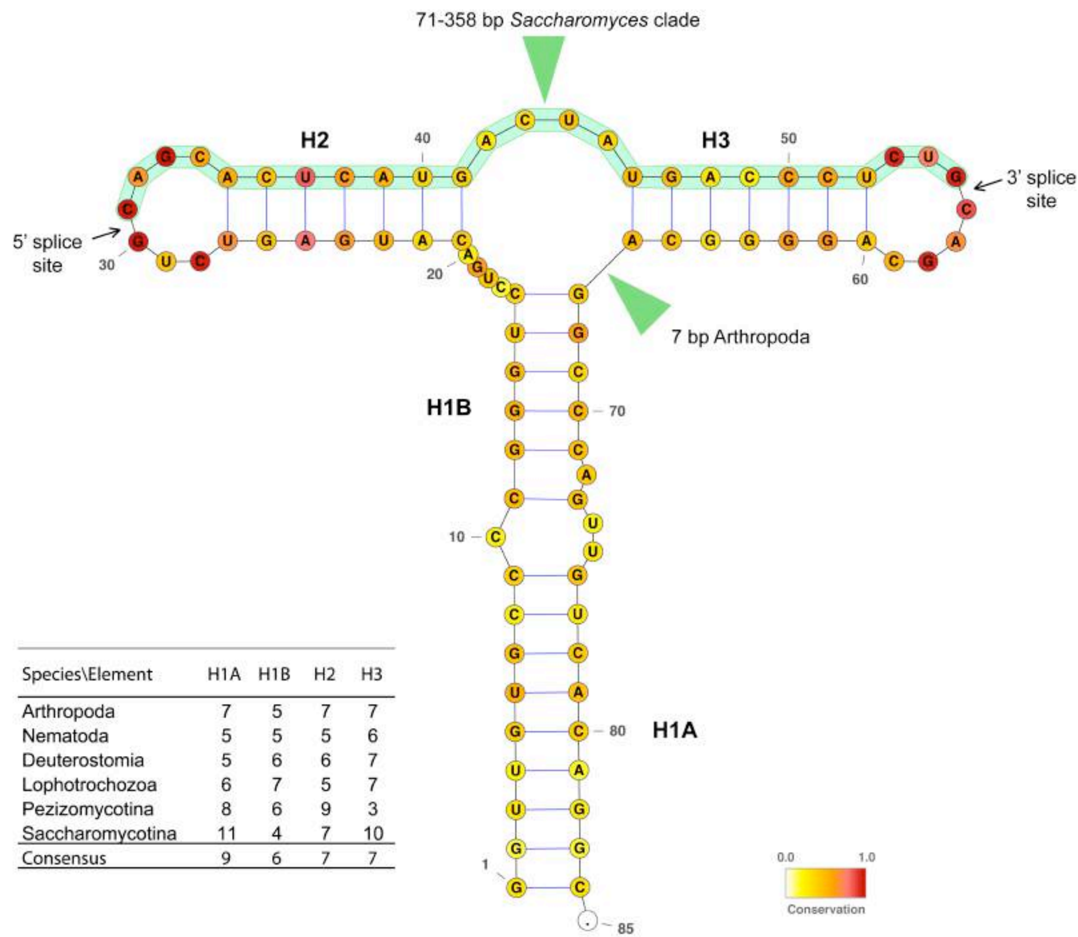


Filipiowicz, EMBO J (2014)

HAC1



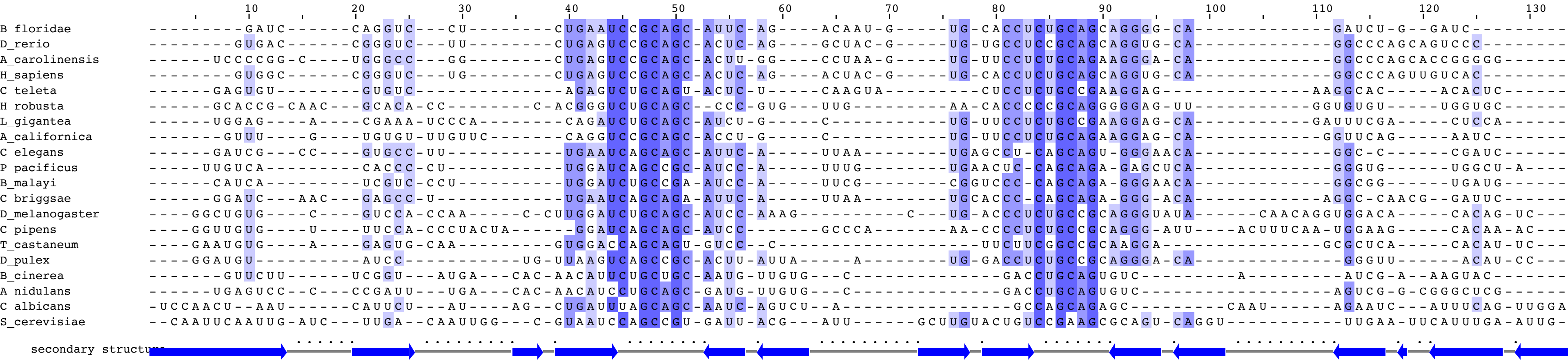
Filipiowicz, EMBO J (2014)

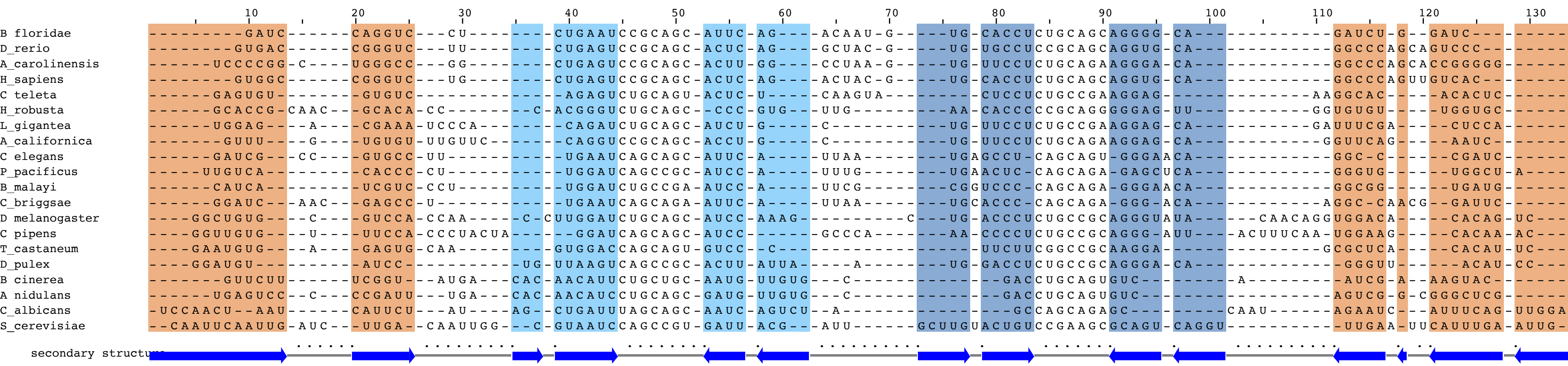


Species\Element	H1A	H1B	H2	H3
Arthropoda	7	5	7	7
Nematoda	5	5	5	6
Deuterostomia	5	6	6	7
Lophotrochozoa	6	7	5	7
Pezizomycotina	8	6	9	3
Saccharomycotina	11	4	7	10
Consensus	9	6	7	7

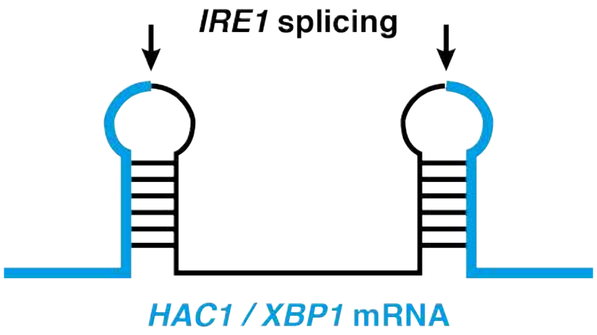
Hooks & Griffiths-Jones, RNA Biol (2011)

HAC1 conservation





RNA structure and introns



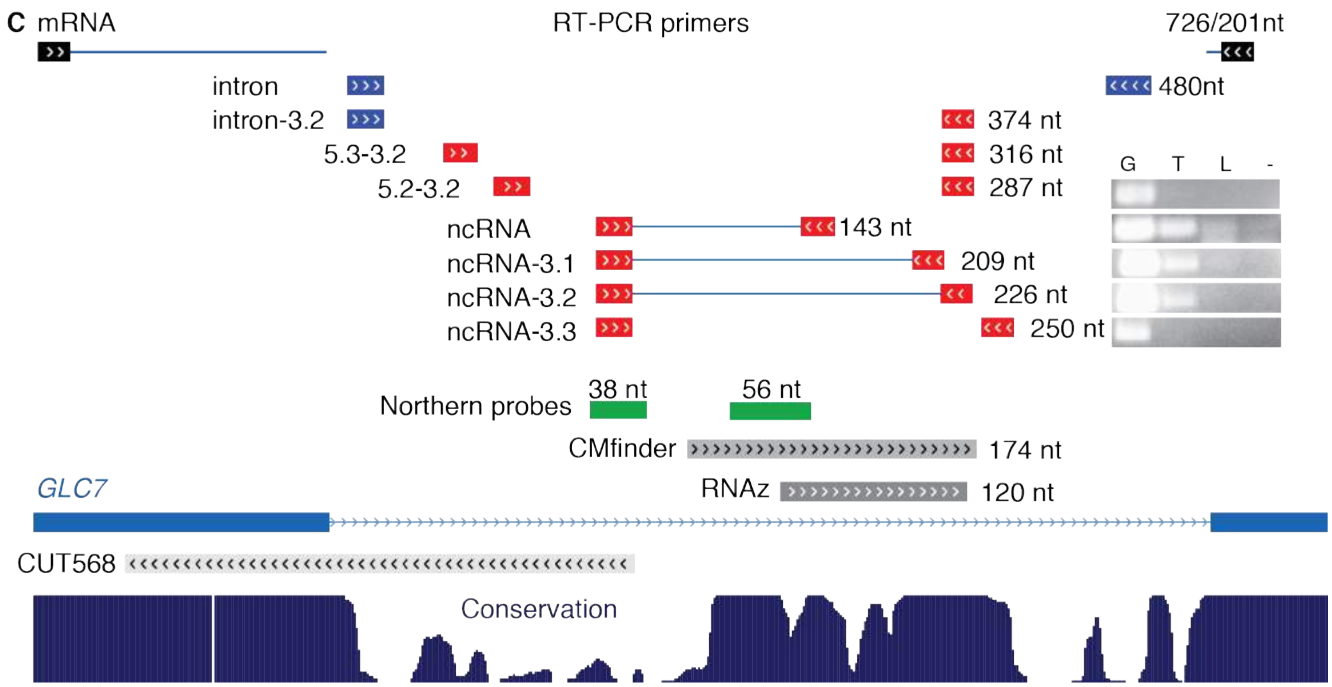
1. *HAC1*

   unconventional splicing

2. *GLC7*

3. *RPL28*

GLC7

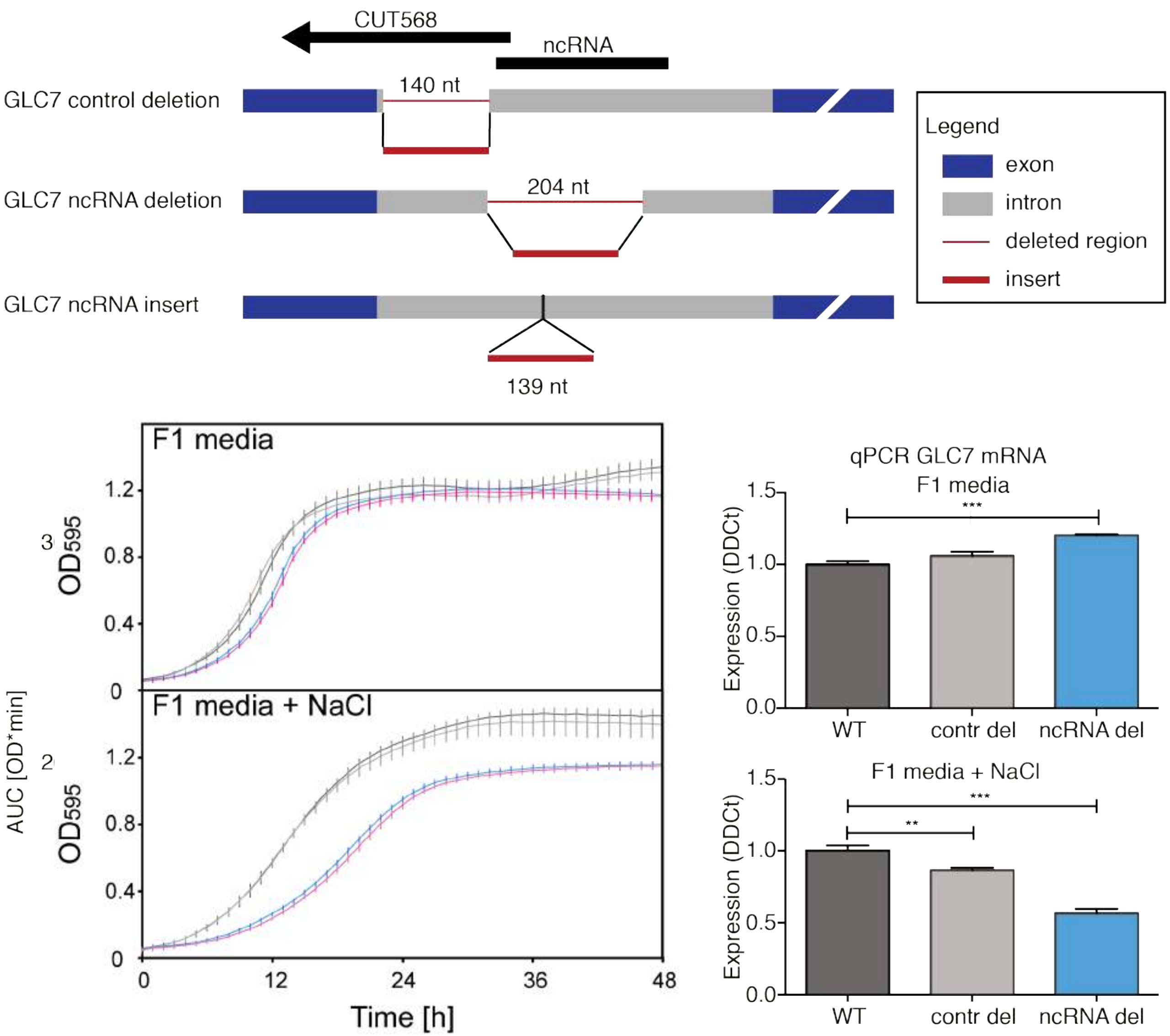


Hooks *et al.* (2016)

GLC7 ncRNA indicated by RT-PCR and the Northern

GLC7 codes for a crucial protein phosphatase catalytic subunit

GLC7 phenotype



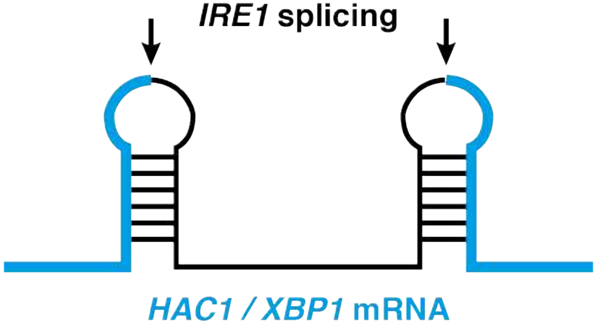
ncRNA deletion mutant is sensitive to salt stress

ncRNA deletion decreases the *GLC7* expression

no rescued by ncRNA expression

Hooks *et al.*, Genetics (2016)

RNA structure and introns



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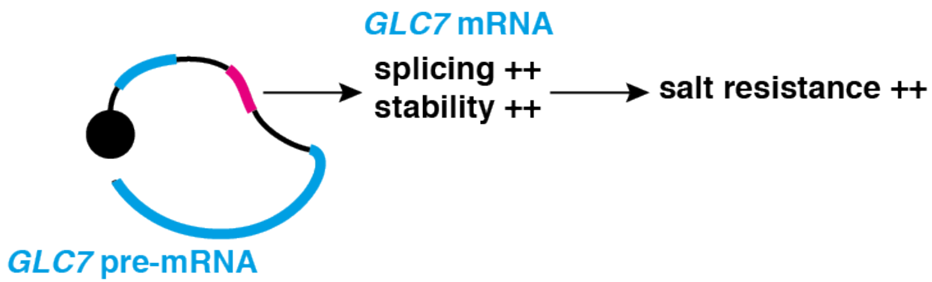
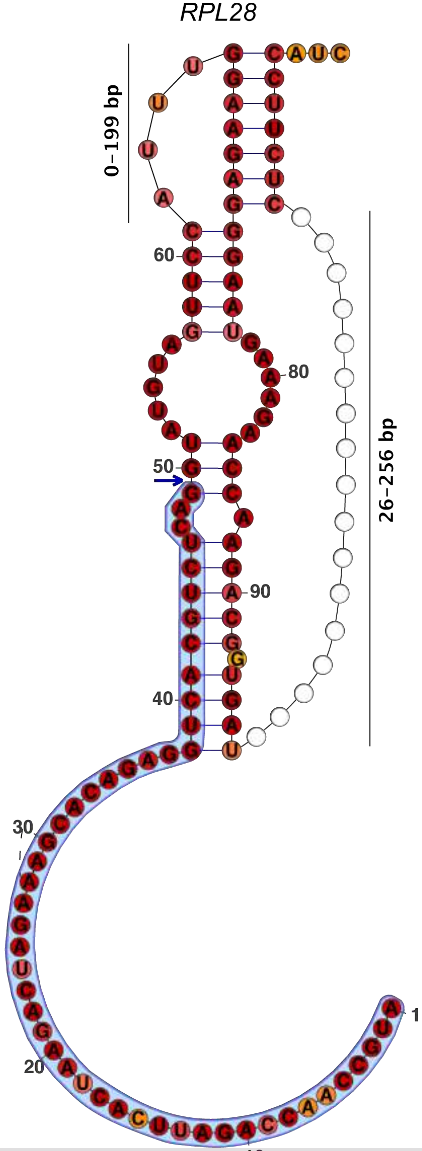


2. *GLC7*

   conveys resistance to salt stress

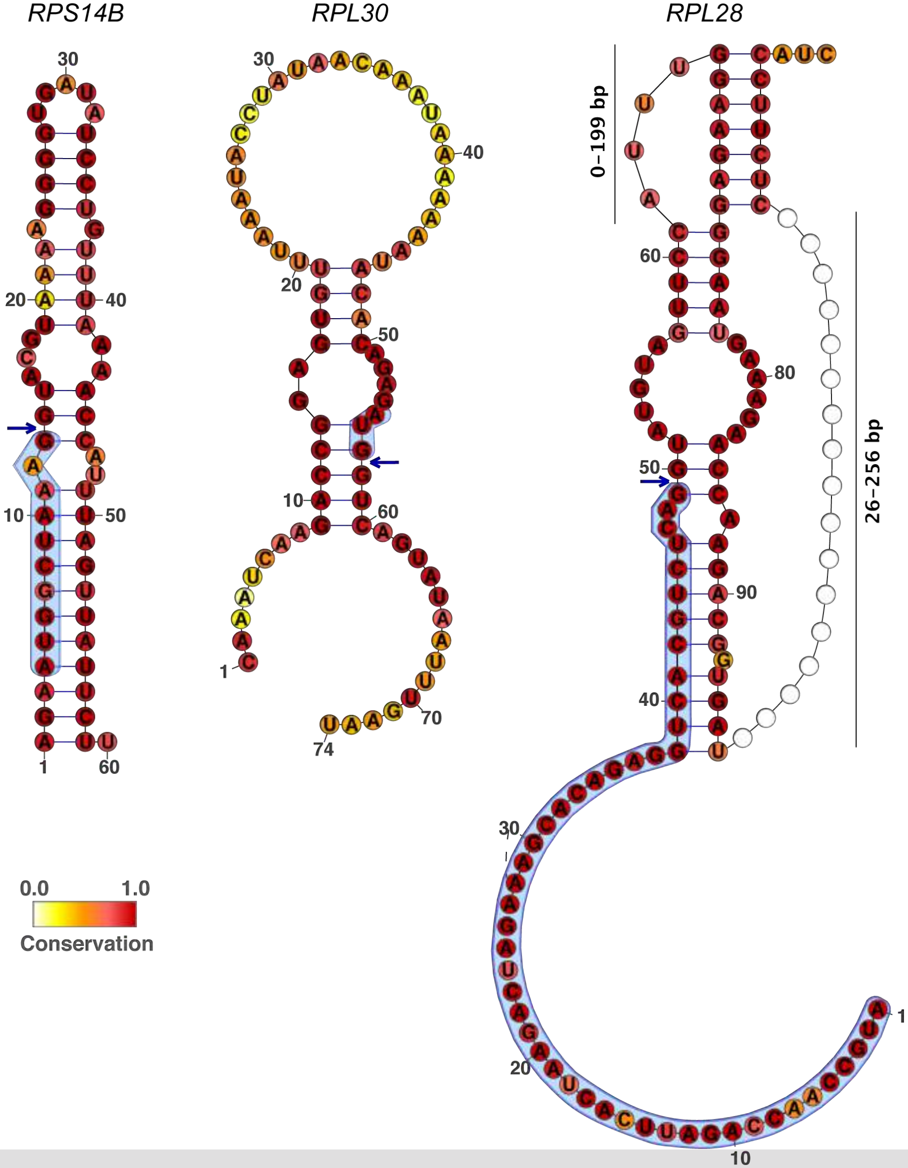
3. *RPL28*

RPL28 structure



- Similar hairpin structures in pre-mRNAs of *RPS14B*, *RPL30* and *RPL28*

RPL28 structure

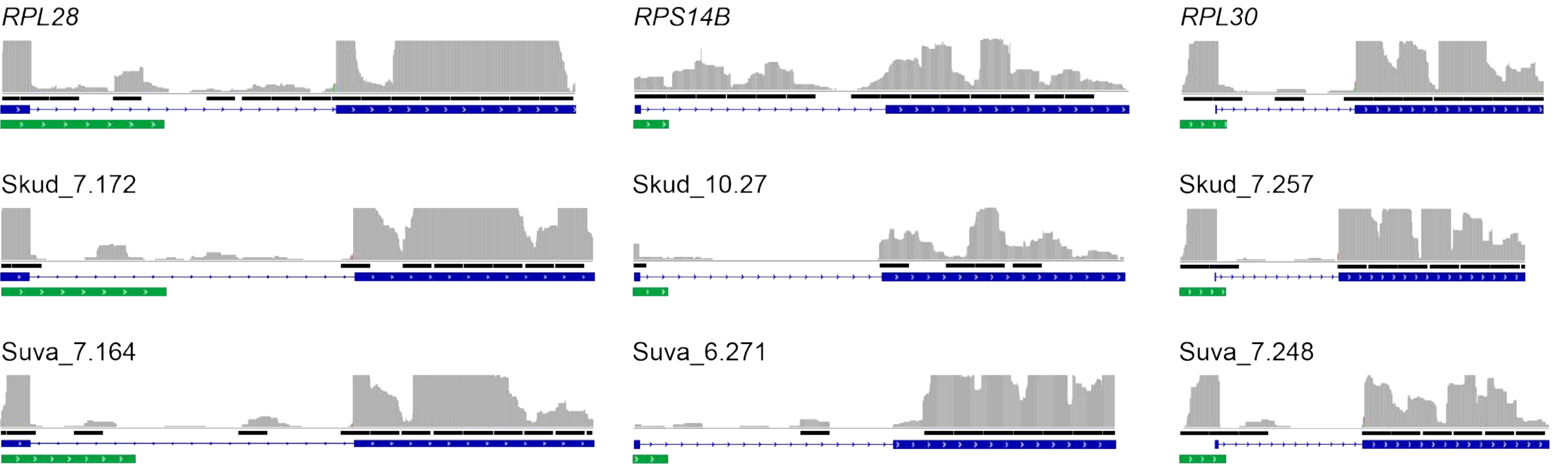


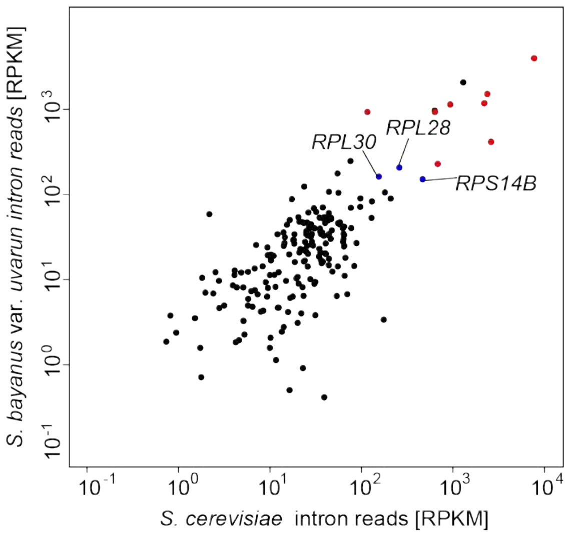
- Similar hairpin structures in pre-mRNAs of *RPS14B*, *RPL30* and *RPL28*

- pre-mRNA structure is recognized by ribosomal proteins with lower affinity than their rRNA targets

- Negative autoregulatory loop

RPL28 expression





Hooks *et al.* (2014)

RPL28, *RPS14B* and *RPL30* are one of the most expressed introns not containing any snoRNA

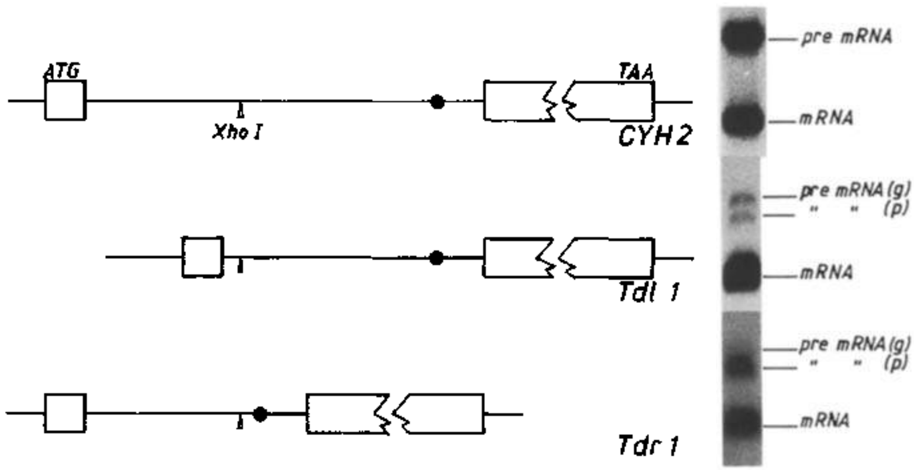
Does RPL28 employ the same mechanism for expression regulation?

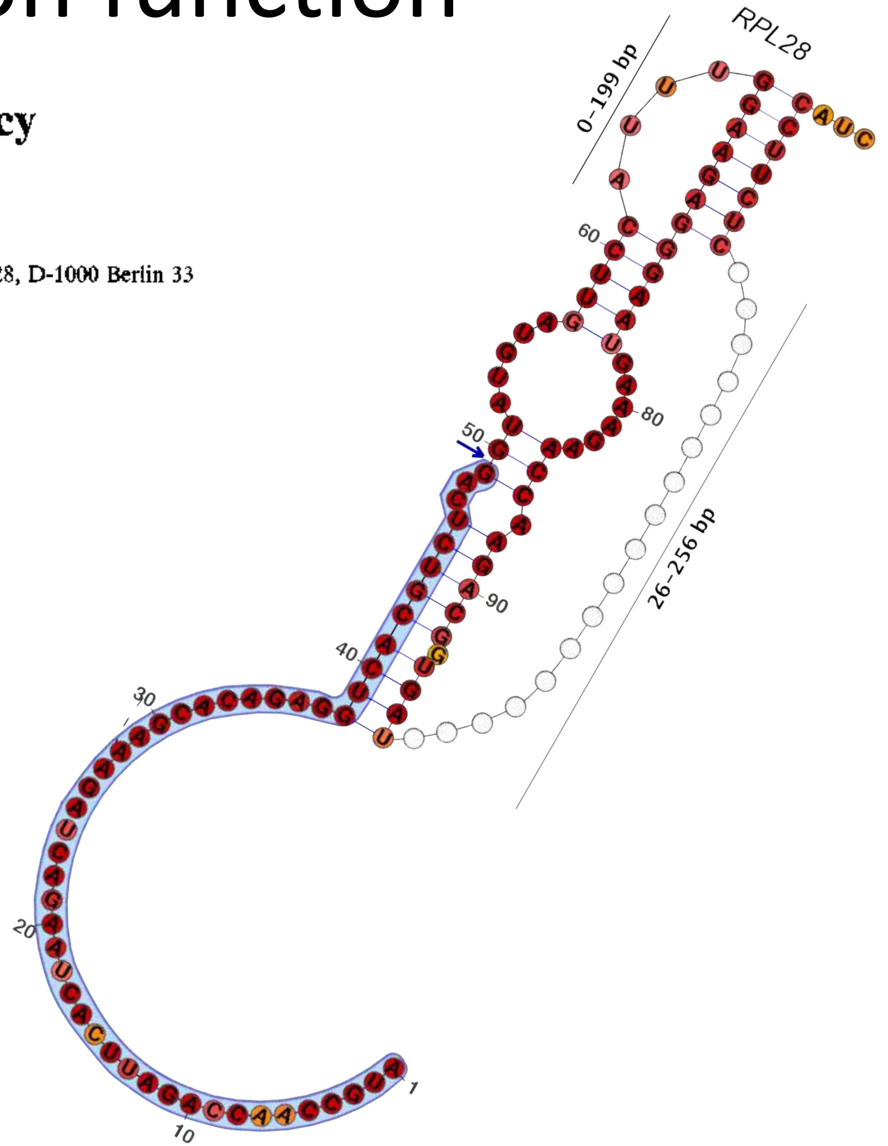
RPL28 intron function

Mol Gen Genet (1986) 203:300–304

Intron mutations that affect the splicing efficiency of the *CYH2* gene of *Saccharomyces cerevisiae*

Ulrike Swida, Eduardo Thüroff, and Norbert F. Käufer*
Institut für Biochemie und Molekularbiologie, Freie Universität Berlin, Ehrenbergstraße 26–28, D-1000 Berlin 33





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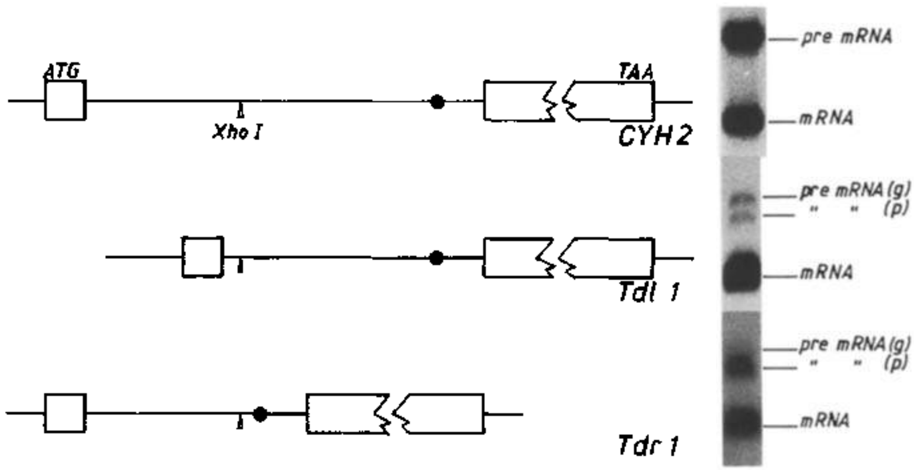
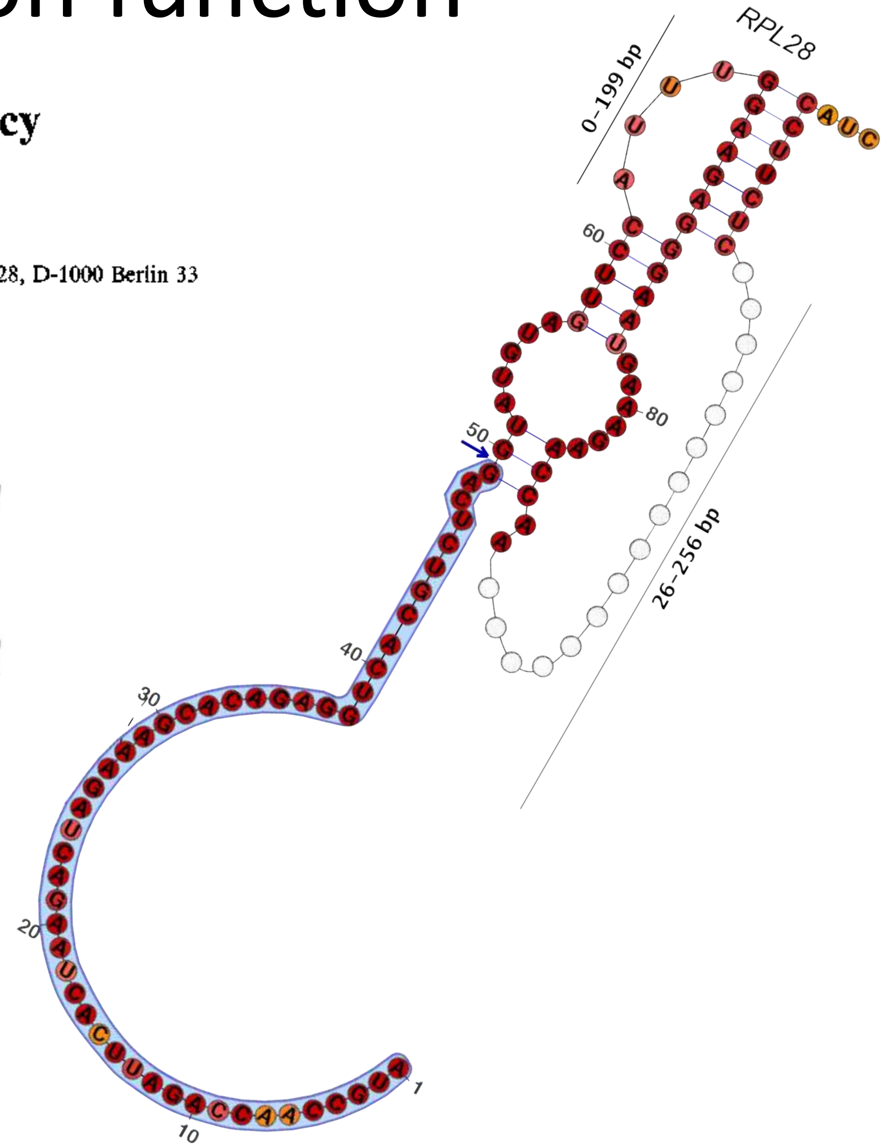
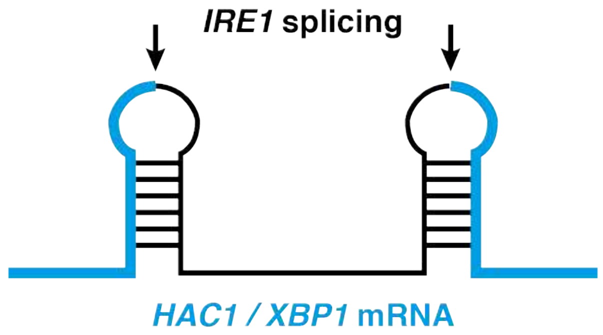




RNA structure and introns



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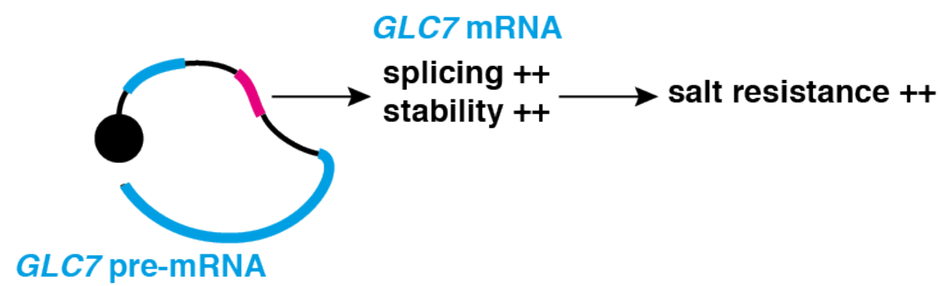
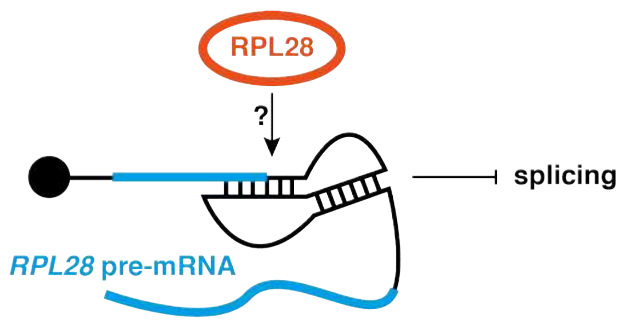


2. *GLC7*

   conveys resistance to salt stress

3. *RPL28*

   hypothesis – RP regulation



Conclusions

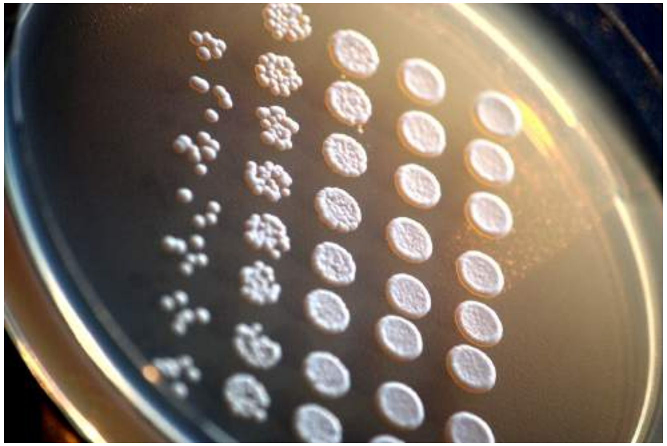


Photo by: Rainis Venta, BY−SA 3.0

- Intron positions are perfectly conserved, sequences are frequently conserved
- Introns are expressed and have a life after splicing
- Introns can perform their function by RNA structures
- Intronic RNAs regulate the transcription of their host genes

Acknowlegments

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