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The RNase III substrate specificity from that the *E. coli's* to the Humans'.

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Graphical Abstract

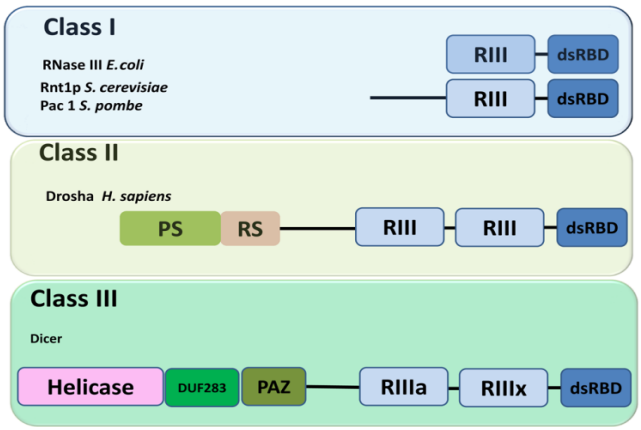

Figure 1. Linear schematic of domain structures of class I–III RNase III proteins. Class I is represented by E. coli RNase III, Rnt1p of S cerevisiae and Pac1 of S.pombe; Class II by mammalian Drosha and Class III by Dicer-like RNases III. Domain key: RIII - RNase III domain; dsRBD - dsRNA-binding domain; PR - proline-rich domain; RS - arginine/serine-rich domain; DUF283-unknown function domain; PAZ-PAZ domain. Diagrams are not to scale

Abstract.

The Ribonuclease III (RNase III) enzymatic family is implied in many important biological processes from the bacteria until humans. In this sense, they have been selected as drug-target candidates for drug development. Although RNase III members show a high degree of sequence diversity, they generally share common structural and some degree of enzymatic activity. The accessory domains are key determinants for both the substrate selectivity and the biological function of each RNase III type, however, their role is still under study. The in vitro enzymatic activity of three RNase III members from class I (E. coli RNase III, Schizosaccharomyces pombe Pac1 and Saccharomyces cerevisiae Rntp1) and the human Drosha placed in class II was tested against four different substrates. These two RNase III classes encompass members having different domain organization. Enzymatic activity differences were found among members of the class I, which get increased when the human Drosha (class II) was included in the test. The substrate promiscuity of the *E. coli* RNase III was corroborated in vivo through its expression in S.

	cerevisiae, as reported previously, but was extended here to *Pichia pastoris*. The new biochemical data integrated with previous studies confirmed that RNases III substrate specificity is highly influenced by its protein structure architecture. This fact also allowed drawing evolutionary links between RNase III members from their structural and substrate specificity differences.

Results

1. The enzymatic activity of the RNases III class I and II

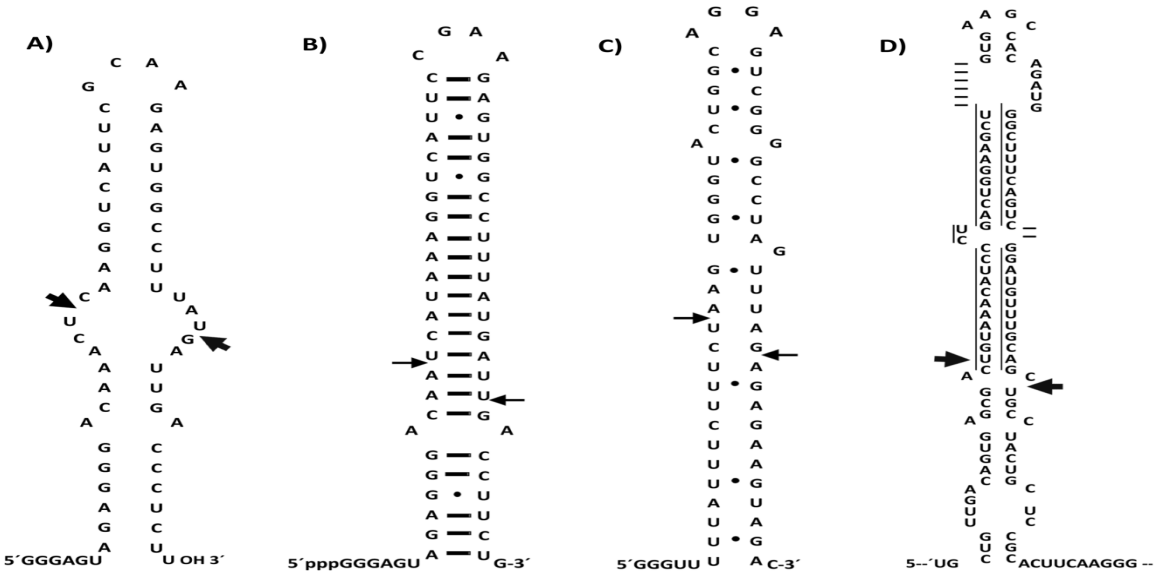

Figure 2. Secondary structures for the four designed dsRNA substrates used for the evaluation of the ribonuclease activity. (A) Authentic *E. coli* RNase III substrate (R1.1). (B) Pac1 substrate obtained from alteration of R1.1, NGNN-capped. (C) Rnt1p substrate with NGNN terminal tetraloop. (D) Drosha substrate, pri-miRNA. Arrows indicate the cleavage site for its corresponding RNases III.

Table 1. *In vitro* enzymatic activity of *E. coli* RNase III, *S. pombe* Pac1, *S. cerevisiae* Rnt1p and human Drosha against optimized dsRNA substrates. All mean values of enzymatic activities were significant regarding the control at p-level <0.05.

Enzymatic dsRNA endonuclease activity 10^5 U/mg (*)				
	109 nt dsRNA *E. coli* RNase III	101 nt dsRNA *S. pombe* Pac1	82 nt dsRNA *S. cerevisiae* Rnt1p	108 nt dsRNA Human DROSHA
E. coli RNase III	6,3883	6,3963	6,1961	6,1971
S. pombe Pac1	6,4350	6,7918	6,7795	6,7795
S. cerevisiae Rnt1p	3,0644	6,6614	6,6544	2,6357
Human Drosha	2,3391	2,9295	2,7669	8,7594
No enzyme	1,3601	1,3047	1,4071	1,3872

*The unit definition for all RNase III types was taken as the amount of enzyme able to solubilize 1 nmol acid precipitable dsRNA per hour (Dunn, 1982). N=9 (Number of replicates per each enzymatic assay)

Our results confirm that the complexity of the structural architecture of the RNases III is closely related to their substrate specificity. The simplest architecture of the *E. coli* RNase III determines its high substrate promiscuity since there is no need of any substrate determinant for the cleavage. However, more complex architectures, like human Drosha (Figure 1) that is evolutionarily quite distinct from the E. coli RNase III, do require specific secondary structures of dsRNAs to carry out the cleavage, having low activity on other substrates behind its own. Differences in RNases III architecture were evidenced by sequence comparative analyses (**Figure 3**)

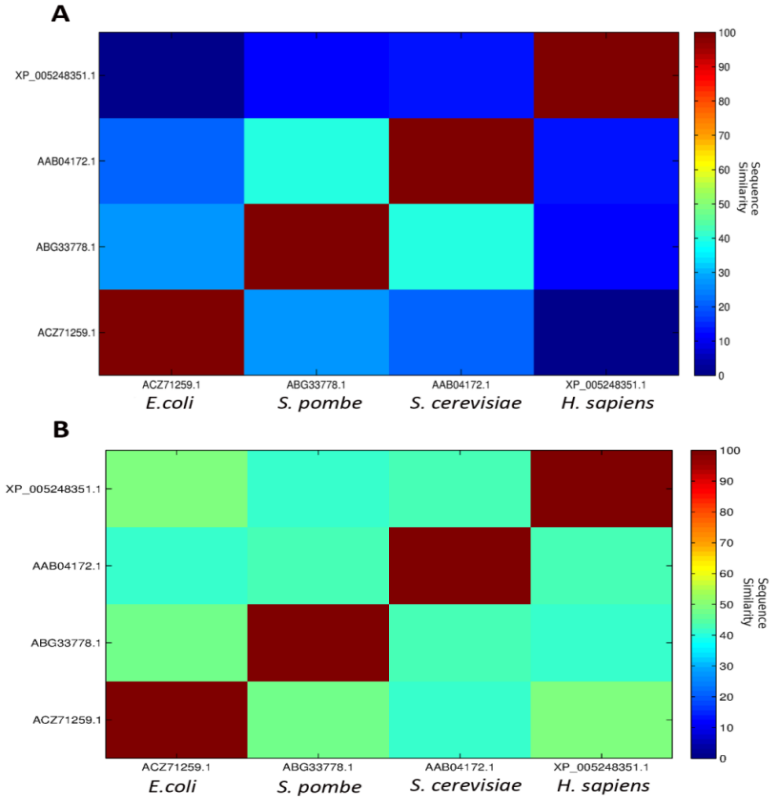

Figure 3. (A) Global similarity matrix expressed in percentage (color bar) for the protein RNase III sequences involved in the study. IDs at GenBank for (**ACZ71259.1:** *E. Coli* RNase III); (**ABG33778.1:** *S. pombe* Pac1); (**AAB04172.1:** *S. cerevisiae* Rnt1p), ID at NCBI for (**XP_005248351.1:** *H. sapiens* Drosha). **(B)** Local similarity matrix expressed in percentage (color bar) for the protein RNase III sequences involved in the study. Same IDs were kept.

2. *E. coli RNase III shows in vitro relaxed substrate specificity over dsRNAs – confirming its lethal effect over two different yeasts species.*

E. coli RNase III enzyme indiscriminately cleaved in vitro all RNA duplexes depicted in **Figure 2**, showing a relaxed substrate specificity which is coherent with its simple structural architecture. These findings are in good agreement with previous in vitro results that show little structural substrate requirements for the E. coli RNase III cleavage [1-3]. Although, E. coli RNase III activity has been deeply studied in vitro, few reports have demonstrated its substrate specificity in vivo [4]. The expression of *E. coli* RNase III in *S. cerevisiae* and its lethal effect on this yeast species have been reported previously by Inouye *et al.* in 1998 [4]. We revisited these experiments aiming to further clarify the lethal effect of the bacterial RNase III expression on the Saccharomycetes class and to assess if the relaxed substrate specificity shown *in vitro* could be followed *in vivo* with diverse heterologous dsRNAs. To evaluate *in vivo* the bacterial RNases III specificity over diverse dsRNAs, the *E. coli* RNase III was expressed in transformed *S. cerevisiae* and *P. pastoris* yeasts. If a catastrophic effect on the metabolism of both yeasts is observed, then it is very likely that the bacterial RNase III relaxed activity shown *in vitro* could take place *in vivo* (**Figure 4)**

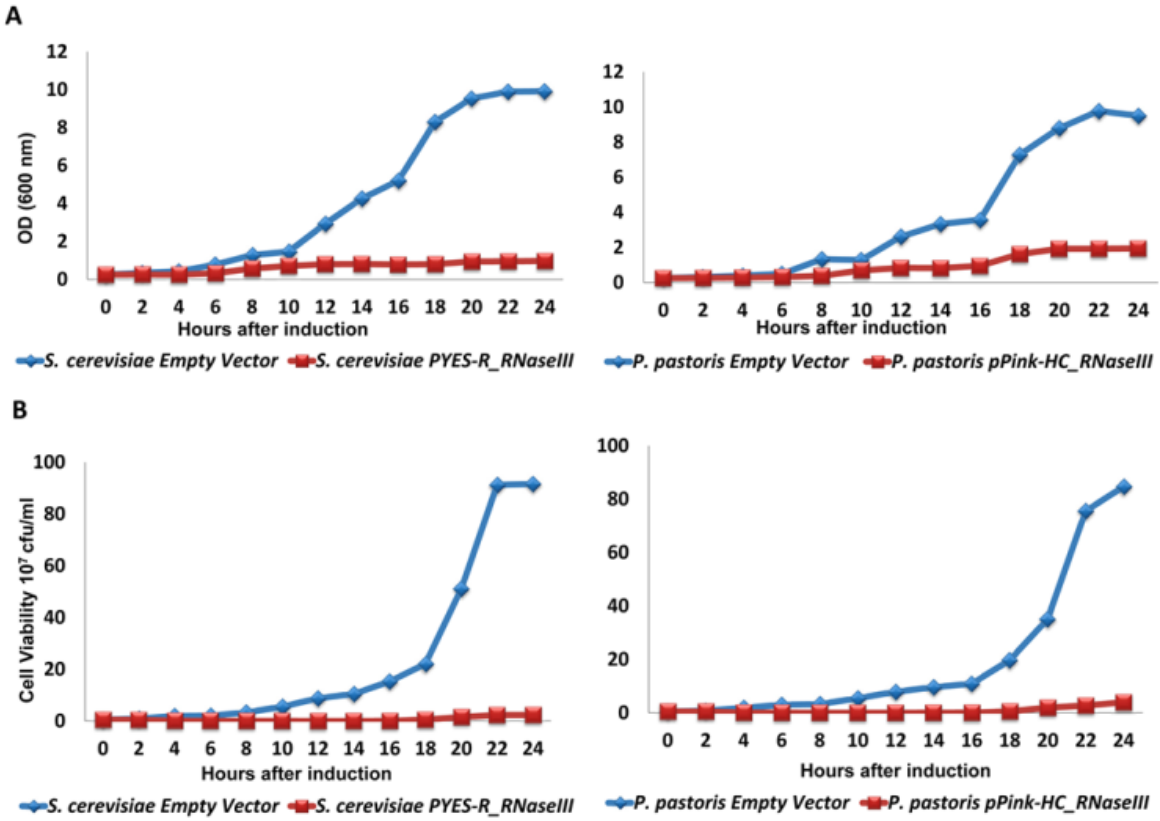

Figure 4. **(A)** Effect of *E. coli* RNase III expression on the growth of transformed yeasts *S. cerevisiae* and *P. pastoris*. Cells growth was followed by measuring optical density at 600 nm each 2 hours until 24 hours after induction. **(B)** Effect of *E. coli* RNase III expression on cell viability of transformed yeasts *S. cerevisiae* and *P. pastoris*. The cell viability was monitored during 24 hours post-induction every 2 hours

References

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[2] Yang, D.; Buchholz, F.; Huang, Z.; Goga, A.; Chen, C.-Y.; Brodsky, F. M.; Bishop, J. M., Short RNA duplexes produced by hydrolysis with Escherichia coli RNase III mediate effective RNA interference in mammalian cells. *Proceedings of the National Academy of Sciences* **2002,** *99* (15), 9942-9947.
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