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

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**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* 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.

**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.
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

![Secondary structures for the four designed dsRNA substrates used for the evaluation of the ribonuclease activity.](image)

**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.

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

*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).

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


