Does the RNA surveillance machinery ensure non-coding RNA integrity?

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Introduction

- RNA surveillance in eukaryotes is essential to ensure the correct responses to stimuli and ensure the correct formation of ribonucleoproteins.
- Non-coding RNAs are directed towards the exosome by the TRAMP complex if they are to be degraded
- The eukaryotic exosome is made up of 9 core proteins that form a barrel and is associated with two nucleases, Rrp6 (an exoribonuclease) and Rpp44 (an endo- and exoribonuclease).
- The TRAMP complex consists of the Trf4 poly(A)polymerase , Air1 or Air2 (homologous RNA binding proteins) and Mtr4, a helicase.
- The TRAMP complex is thought to polyadenylate RNA substrates to enhance their degradation.
- Recent work shows that mutants lacking components of the RNA surveillance machinery lead to aberrantly processed scR1, the RNA component of the Signal Recognition Particle in *S.cerevisiae.*
- Whether the RNA surveillance machinery plays a role in other ncRNA processing is unknown.

Aims

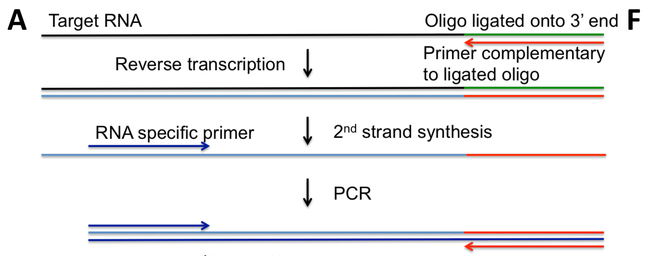
- Enrich ribonucleoproteins from *S. cerevisiae* strains lacking RNA surveillance machinery components (*trf4Δ* and *air1air2Δ*) to provide a pool of ncRNAs for sequencing.
- Perform ligation mediated reverse transcriptase PCR on various non-coding RNAs (ncRNAs)
- Sequence PCR products to determine whether the RNA surveillance machinery plays a role in the integrity of ncRNAs.

RNP Enrichment

- 2 strategies were used to enrich RNPs, with the aim of generating a pool of RNA from these complexes for next-generatioin sequencing. These were 10%-30% glycerol gradients and aminobutyl agarose columns.

Using RT-PCR to examine 3’ sequences

- While the RNP enrichment was being developed, several specific RNAs were amplified from total RNA pools using an oligonucleotide ligation RT-PCR methodology previously used to examine scR1 (Figure A). This was to determine if other RNAs may be affected by loss of RNA surveillance factors.
- Purified PCR products were sequenced to determine the 3’ end of the target RNA.



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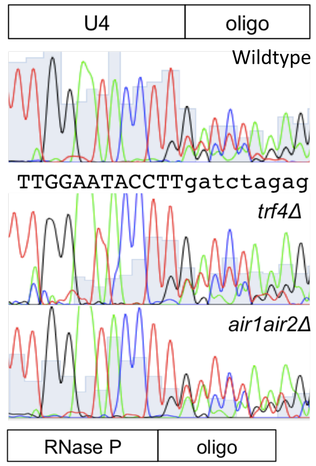
RNA	Strain	3' end sequence
SCR1	Database	CATATTTTT
	W/T	CATATTTTT
	Trf4Δ	CATATTTTT
	air1air2Δ	AACCATAT
U1	Database	ATTTATTTT
	W/T	CTTTATTTT
	Trf4Δ	NNTTATTTT
	air1air2Δ	NNTTATTTT
U4	Database	GAATACCTTT
	W/T	GAATACCTT
	Trf4Δ	GAATACCTT
	air1air2Δ	GAATACCTT
U5-S	Database	TTTGGAACT
	WT	GGAACTTTT
	Trf4Δ	GGAACTTTT
	air1air2Δ	GGAACTTTT
U5-L	Database	GGAGGGCGT
	W/T	GGAGGGAGT
	Trf4Δ	TGAGGGAGT
	air1air2Δ	TGAGGGAGT
RNase P	Database	TGTTCCAGC
	W/T	TGTTCCAGC
	Trf4Δ	TGTTCCAGC
	air1air2Δ	TGTTCCAGCCCATATCCAACTTCCAAT

G



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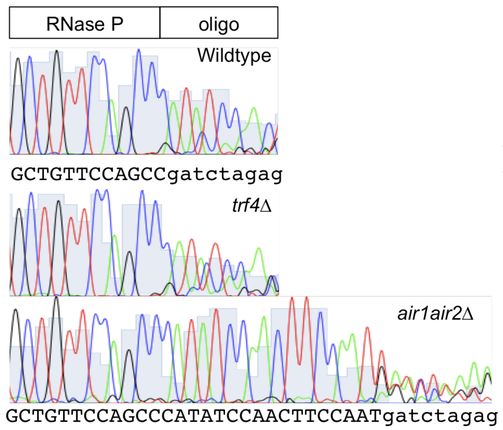

Results

RNP enrichment:

- Glycerol gradient sedimentation successfully isolated scR1, snR42 (Figures B, D) and U1 snRNA (not shown) within the gradient, while ribosomes pelleted (not shown).
- Ribonucleoproteins bind to aminobutyl agarose at low salt (50 mM KOAc), and when cell extracts were incubated with the resin scR1, snR42 and U1 snRNA were removed from the flow through. High (0.8 M KOAc) salt was used successfully to elute the RNPs (Figures C and E, and data not shown).
- The RNA isolated on columns (e.g. Fig. C lanes 1 -3) was more intact than that from gradient fractions (e.g. Fig. B lanes 5-8).

RT-PCR:

- The RNAs chosen for analysis are all processed to the mature forms from longer precursor transcripts.
- Sequencing products of U1, U4 and U5 snRNA were similar in wild type cells and those lacking components of the TRAMP complex.
- Strains lacking the Air1 and Air2 components of TRAMP show partial processing of RNase P RNA.

Conclusions

- The RNA surveillance machinery does not seem to play a role in the integrity of U1, U4 and U5.
- RNase P seems to be partially processed without Air1 and Air2, suggesting the TRAMP complex playing a role in its processing.

Future work

- Investigate the role of the RNA surveillance machinery further for U2, U3, U6, RNase MRP and telomerase RNA.
- Use deep sequencing to get information on heterogeneous populations in mutants.
- Investigate mechanism of partially processed RNase P transcript .

Acknowledgements

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References

- Leung et al (2014), Nucleic Acids Research, 42 (16), 10698-10710
- Schmidt & Butler (2013), Wiley International Reviews: RNA, 4 (2), 217-231

A Diagram of RT-PCR process **B-E** Extracts were prepared from the indicated strains and either fractionated on 10-30% v/v glycerol gradients (B and D) or incubated with amino butyl agarose under low salt (50 mM KOAc) conditions, with bound material being eluted with high salt (0.8 M KOAc) (C and E). RNA was prepared from gradient fractions or elusions from resin, and subjected to Northern analysis using probes against the RNAs indicated **F** Summary of 3’ end sequence analysis data **G H** Chromatograms of Sanger sequencing data for U4 snRNA and RNase P RNA from wildtype, *trf4Δ* and *air1air2Δ* strains.

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