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Abundance of tRNA-derived small RNAs in phosphate-starved Arabidopsis roots

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Several research advances have indicated an important role of transfer RNA (tRNA)-derived small RNAs in modulating developmental processes or stress responses. Recently, from the deep sequencing of small RNAs in Arabidopsis (*Arabidopsis thaliana*), we identified a new class of 19-nucleotide (nt) small RNAs corresponding to the 5' end of tRNA accumulated at high levels in phosphate-starved roots. In two very recent studies, 19-nt tRNA fragments were also observed in human cells, suggesting their widespread nature. In our study, tRNA halves cleaved at the anticodon loop, the most common tRNA fragments found, were predominant in roots. These results showed a spatial and temporal expression pattern of small RNAs derived from specific cleavage of tRNA molecules. Although the function of these tRNA-derived small RNAs under phosphate deficiency remains unknown, their diversity, biogenesis and potential function are henceforth summarized and discussed. Certainly, they will emerge as a novel class of regulatory small RNAs.

Key words: tRNA, small RNA, deep sequencing, phosphate deficiency

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Transfer RNAs (tRNAs), a fundamental component of the translation machinery, convert the message encoded in nucleic acids into proteins through ribosome-dependent protein synthesis.[1] In addition to their canonical roles in protein synthesis, tRNAs are increasingly suggested to have an additional role in modulating developmental processes or stress responses, because their specific cleavage occurs in many organisms, predominantly in specific tissues or under stress conditions such as oxidative stresses and starvation (reviewed in ref. 2).

In this study,[3] we used Solexa high-throughput sequencing technology to obtain a large population of small RNA sequence reads from root and shoot tissues of Arabidopsis (*Arabidopsis thaliana*) under phosphate-sufficient or -deficient conditions. The use of this strategy allowed for a genome-wide analysis of Arabidopsis small RNAs in response to phosphate deficiency. Strikingly, we found a new class of 19-nt small RNAs corresponding to the 5' end of tRNA accumulated at a high level, up to 34.32% of total small RNA reads, in phosphate-starved roots. The abundance of these small RNAs from specific tRNA species is not associated with codon usage bias. This class of tRNA-derived small RNAs is somewhat unique because they differ in nucleotide number from the tRNA halves (30–40 nts) cleaved at the anticodon loop in most reported cases. Two recent studies also identified such 19-nt tRNA-derived small RNAs from human cells.[4,5] These studies not only support our finding, but also point toward a universal and important role for this class of tRNA fragments. In addition to revealing the 19-nt small RNAs, our RNA gel blot analyses showed the preferential accumulation of tRNA halves in roots compared to shoots.[3]

Rny1 and angiogenin are 2 nucleases responsible for the cleavage of tRNA molecules in yeast[6] and mammalian cells,[7,8] respectively. By searching the Arabidopsis genome, several genes showing homology

Table 1. Summary of knowledge of tRNA-derived small RNAs in different organisms

Organism	Length (nt)	Cleavage			Function[a]	Reference
		Positions	Nuclease[a]	Conditions/Tissues or cells		
Microbes						
Streptomyces coelicolor	30–35	anticodon loop	-	starvation/aerial hyphae	-	13
Aspergillus fumigatus	36–39	anticodon loop	-	conidiogenesis	-	14
Giardia lamblia	44–49	anticodon loop, variable loop	-	starvation/temperature	-	15
Saccharomyces cerevisiae	35–50	anticodon loop	Rny1	stationary phase entry, heat shock, nitrogen starvation, methionine starvation, oxidative stress	-	6, 16
Tetrahymena thermophila	33–42	anticodon loop, variable loop	-	amino acid starvation	-	17
Insects						
Drosophila melanogaster	16–29	not reported	-	developmental processes	-	18
Plants						
Cucurbita maxima (pumpkin)	31–68	anticodon loop, D loop		phloem sap	inhibit protein translation	12
Arabidopsis thaliana	48–55	anticodon loop	-	oxidative stress	-	16
	19, 30–40	D loop, anticodon loop	-	phosphate starvation/root tissues	-	3
Mammals						
Cercopithecus acthiops (green monkey)	31–38	anticodon loop	-	Cos7 cell line	-	7
Homo sapiens (human)	35–45	anticodon loop	-	oxidative stress/HeLa cell line	-	16
	31–38	anticodon loop	angiogenin	nutrition deficiency, heat shock, hypothermia, hypoxia	-	7
	30–45	anticodon loop	angiogenin	arsenite, heat shock, UV irradiation/U2OS cell line	-	8
	19–25, 30–39	anticodon loop, T loop	-	various cell lines and tissues	-	19
	19	D loop	DCL1	HeLa cell line	-	4
	17–26	D loop, T loop, 3' end of precursor	ELAC2	proliferating cancer cells	cell proliferation	5

[a]Undetermined nucleases and functions are indicated as "-".

with Rny1 can be identified. Like Rny1, these genes all belong to the RNase T2 family. Interestingly, two of the genes, RNS1 and RNS2, were reported to be upregulated by phosphate starvation.[9-11] Moreover, Dicer 1 was reported to be involved in producing the 19-nt tRNA-derived small RNAs in HeLa cells.[4] Identification of the corresponding ribonucleases in plants will uncover the biogenesis of these small tRNA fragments and may provide insights into their function.

Despite widespread observations of these tRNA-derived small RNAs, their biological function is not yet clear.[2] tRNA-derived small RNAs may be involved in the inhibition of protein translation[8,12] or function like siRNAs or miRNAs.[2,4] A recent report demonstrated that an RNA fragment derived from the 3' end of a tRNA precursor transcript not retained in the mature tRNA is required for cell proliferation.[5] Discovery of such tRNA fragments has added additional complexity to the inventory of tRNA-derived small RNAs. In **Table 1**, we summarize current knowledge of tRNA-derived small RNAs in terms of biogenesis, characteristics and potential function. Their biological significance is expecting to be revealed in the near future.

References

1. Giege R. Toward a more complete view of tRNA biology. Nat Struct Mol Biol 2008; 15:1007-14.
2. Thompson DM, Parker R. Stressing out over tRNA cleavage. Cell 2009; 138:215-9.
3. Hsieh LC, Lin SI, Shih AC, Chen JW, Lin WY, Tseng CY, et al. Uncovering small RNA-mediated responses to phosphate deficiency in Arabidopsis by deep sequencing. Plant Physiol 2009; 151:2120-32.
4. Cole C, Sobala A, Lu C, Thatcher SR, Bowman A, Brown JW, et al. Filtering of deep sequencing data reveals the existence of abundant Dicer-dependent small RNAs derived from tRNAs. RNA 2009; 15:2147-60.
5. Lee YS, Shibata Y, Malhotra A, Dutta A. A novel class of small RNAs: tRNA-derived RNA fragments (tRFs). Genes Dev 2009; 23:2639-49.
6. Thompson DM, Parker R. The RNase Rny1p cleaves tRNAs and promotes cell death during oxidative stress in *Saccharomyces cerevisiae*. J Cell Biol 2009; 185:43-50.
7. Fu H, Feng J, Liu Q, Sun F, Tie Y, Zhu J, et al. Stress induces tRNA cleavage by angiogenin in mammalian cells. FEBS Letts 2009; 583:437-42.

8. Yamasaki S, Ivanov P, Hu G-f, Anderson P. Angiogenin cleaves tRNA and promotes stress-induced translational repression. J Cell Biol 2009; 185:35-42.
9. Taylor CB, Bariola PA, delCardayre SB, Raines RT, Green PJ. RNS2: a senescence-associated RNase of Arabidopsis that diverged from the S-RNases before speciation. Proc Natl Acad Sci USA 1993; 90:5118-22.
10. Bariola PA, Howard CJ, Taylor CB, Verburg MT, Jaglan VD, Green PJ. The Arabidopsis ribonuclease gene *RNS1* is tightly controlled in response to phosphate limitation. Plant J 1994; 6:673-85.
11. Bariola PA, MacIntosh GC, Green PJ. Regulation of S-like ribonuclease levels in Arabidopsis. Antisense inhibition of *RNS1* or *RNS2* elevates anthocyanin accumulation. Plant Physiol 1999; 119:331-42.
12. Zhang S, Sun L, Kragler F. The Phloem-delivered RNA pool contains small noncoding RNAs and interferes with translation. Plant Physiol 2009; 150:378-87.
13. Haiser HJ, Karginov FV, Hannon GJ, Elliot MA. Developmentally regulated cleavage of tRNAs in the bacterium *Streptomyces coelicolor*. Nucl Acids Res 2008; 36:732-41.
14. Jochl C, Rederstorff M, Hertel J, Stadler PF, Hofacker IL, Schrettl M, et al. Small ncRNA transcriptome analysis from *Aspergillus fumigatus* suggests a novel mechanism for regulation of protein synthesis. Nucl Acids Res 2008; 36:2677-89.
15. Li Y, Luo J, Zhou H, Liao J-Y, Ma L-M, Chen Y-Q, et al. Stress-induced tRNA-derived RNAs: a novel class of small RNAs in the primitive eukaryote *Giardia lamblia*. Nucl Acids Res 2008; 36:6048-55.
16. Thompson DM, Lu C, Green PJ, Parker R. tRNA cleavage is a conserved response to oxidative stress in eukaryotes. RNA 2008; 14:2095-103.
17. Lee SR, Collins K. Starvation-induced cleavage of the tRNA anticodon loop in *Tetrahymena thermophila*. J Biol Chem 2005; 280:42744-9.
18. Aravin AA, Lagos-Quintana M, Yalcin A, Zavolan M, Marks D, Snyder B, et al. The small RNA profile during *Drosophila melanogaster* development. Dev Cell 2003; 5:337-50.
19. Kawaji H, Nakamura M, Takahashi Y, Sandelin A, Katayama S, Fukuda S, et al. Hidden layers of human small RNAs. BMC Genomics 2008; 9:157.