RNA BIOLOGY
2020, VOL. 17, NO. 8, 1057–1059
https://doi.org/10.1080/15476286.2020.1785196

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EDITORIAL

The regulatory world of tRNA fragments beyond canonical tRNA biology

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Research on tRNA had its roots in the late 1950s and we all have meanwhile learnt quite a lot about tRNA molecules and their universal role during protein biosynthesis, their three-dimensional architecture, their genetics and biogenesis, as well as about their decoration with multiple post-transcriptional modifications. Yet, there is more to be revealed. In the recent past, tRNA-derived fragments have been uncovered in many transcriptome studies in organisms spanning all three domains of life. Since subsequent dedicated functional studies have demonstrated a plethora of regulatory roles for these tRNA fragments, we think it is safe to say that we have witnessed the birth of a new field in RNA biology.

First reports of tRNA-derived fragments have been published in 1977 [1], but at that time, they were not considered molecules possessing physiological roles, rather meaningless degradation byproducts of tRNA homoeostasis. High-throughput sequencing techniques in the last years fostered the discovery of an unforeseen variety of small non-protein-coding RNAs (ncRNAs), including tRNA-derived RNA fragments and has inspired scientists around the world to uncover their functional role(s). As every new field in science, a unifying nomenclature and standardized classification lags behind. Most frequently stable tRNA cleavage products are referred to as tRNA-fragments (tRFs), tRNA-derived RNAs (tdRs), small tRNA-derived RNAs (tsRNAs) or tRNA-derived stress-induced RNAs (tiRNAs). In the context of this *Editorial,* we will use the abbreviation tdR for embracing all forms of tRNA-derived cleavage products which include tRNA halves (size 30–35 nt), and shorter fragments (~14-26 nt) deriving from the 5′, the 3′ or from internal regions of tRNAs, as well as tdRs containing leader or trailer sequences of pre-tRNA molecules. While the first tdRs were discovered in cells exposed to different stress conditions, subsequent studies have shown that some are also constitutively expressed in several cells and tissues, thus probably fulfiling housekeeping roles [2,3].

In our view, tdRs have rightfully secured a place on the ever-growing list of ncRNA regulators playing key roles in RNA biology and disease. Unlike other regulatory ncRNA classes, such as miRNA that execute their biological role in a highly homologous manner, tdRs are functionally quite heterogeneous. tdRs have been implicated in governing transcription, translation, ribosome biogenesis, stress granule formation, apoptosis, cell proliferation, retrotransposition, vesicle-mediated intercellular communication or intergenerational inheritance (and this list is by no means exhaustive). Another clear difference of tdRs compared to miRNAs is the fact that the former are not restricted to one domain of life since they have been found in archaea, bacteria, as well as in uni- and multicellular eukarya [4–6].

We are fascinated by the fact that a single phosphodiester cleavage in the 'mother molecule' of tdRs, genuine tRNA that is, can give rise to processing products with such multifaceted cellular roles. With this Special Focus on 'The regulatory world of tRNA fragments beyond canonical tRNA biology', we try to catch the momentum of tdR research and hopefully give a stimulating overview on this dynamic research field which will provide an important resource for the RNA community.

This Special Focus harbours reports from 15 research groups providing either review papers or genuine research articles. The covered topics of the compiled articles adequately reflect the functional heterogeneity of tdR biology but also highlight our current limitations in comprehensively understanding tdR biogenesis and physiology. Generation of tdRs is a relatively simple process depending on the action of specific endoribonucleases on tRNAs. One of the best understood pathways on the generation of tdRs relies on the action of the ribonuclease angiogenin (ANG), a member of the RNase A superfamily [7,8]. Under stress, ANG is activated to target mature cytoplasmic tRNAs generating two tRNA halves. The 3′-end of the 5′ cleavage product contains a 2′-3′-cyclic phosphate (cP), a feature that can be used to capture and analyse a repertoire of cP-containing tdRs. The Kirino lab used cP-based RNA-seq approaches to show that production of multiple tdRs (and other cP-RNAs) is upregulated under oxidative stress revealing a hidden RNAome layer that is produced in a stress-dependent manner [9].

There is a significant accumulation of available large-scale transcriptome data on the presence of tdRs in different organisms, tissues or disease states. Ideally, such information could be used to predict the functions and mechanisms of tdRs actions towards their putative cellular targets. One of such mechanisms may rely on interactions based on the complementarity between tdRs and their RNA targets as it is employed by the well-understood RNAi machinery. Indeed, binding of tdRs to Argounautes and their loading into RISC complexes is experimentally proven at least in *in vitro* studies.

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Guan et al. used large-scale meta-analyses of available experimental data to predict specific target interactions of selected tdRs and to show that similarly to microRNAs, tdRs can target 3′-Untranslated Regions (UTRs) of mRNAs. Moreover, these analyses showed putative interactions between tdRs and ncRNAs and mRNA introns [10].

Various tdRs show differential expression in healthy and disease states. Yao et al. developed an integrative database, OncotRF, for *in silico* analyses of tdRs across multiple human cancers. The pipeline used in the database allows tdR identification and quantification with the following prediction of their interactions with specific genes or gene networks as well as with clinical outcomes such as survival analyses. Such database is a valuable resource for potential functional annotation of specific cancer-related tdRs and their use as prognostic and diagnostic cancer biomarkers [11].

It is known that specific pools of tRNAs are more commonly targeted by specific RNases. Generation of tdRs is a regulated process, where RNA modifications within tRNAs play important roles in the determination of the efficiency and specificity of cleavage by RNases [12,13]. Rashad et al. show that m1A demethylase Alkbh1 promotes tRNA cleavage in a stress-specific manner. Specifically, they show that the demethylating activity of the enzyme directly contributes to the efficiency of tRNA cleavage [14]. RNA modifications in tRNAs are extremely abundant serving different structural and functional roles [15]. Naturally, tdRs are also enriched for modifications. Although a number of functional studies suggest multiple mechanisms of tdR regulation of gene expression, most of them are based on the use of synthetic tdR molecules lacking natural RNA modifications. The Schaefer and the Ivanov labs provide scalable, simple and cost-effective biochemical approaches to isolate natural endogenous tdRs suitable for mechanistic studies, such as interaction with proteins [16], or structural and functional analyses [17].

While tdRs can regulate gene expression on multiple levels, targeting the translational machinery is one of the well-understood mechanisms of their action [18–20]. In addition to RNAi-like mechanisms, tdRs can bind ribosome or translation factors directly to interfere with their functions. Studies from the Polacek lab show that a specific tdR derived from $tRNA^{Pro}$ binds and associates with mammalian ribosomes and polysomes causing global translational repression in *in vitro* and *in vivo* settings, which correlates with the formation of a low molecular weight translational product. This product is a peptidyl-tRNA formed as a result of translational stalling of ribosomes upon binding of the tdR [21]. In another study, the production of tdRs in *Arabidopsis* depends on the environmental and developmental conditions. Lalande et al. show that a subset of plant tdRs potently inhibits translation. Such protein synthesis inhibition does not require complementarity to the translated mRNA but requires its association with polysomes [22].

Cleavage of tRNAs produces tdRs that can be found both intracellularly and extracellularly. In the review article from Tosar and Cayota, the authors discuss origins and possible functions of tdRs in the extracellular space found in fractions of extracellular vesicles, lipoproteins and ribonucleoprotein complexes [23]. In a related study, Gambaro et al. show that specific tdRs, such as the one deriving from $tRNA^{Gly}$, can be sorted to extracellular vesicles and delivered to recipient cells. They show that intrinsic stability of RNAs (e.g., resistance to nucleases) is a key prerequisite for both maintenance of high tdR concentration intracellularly and the sorting into extracellular vesicles and delivery [24].

Recent data suggest that the composition of tdRs is cell type- and tissue-specific [25]. Su et al. investigated the composition of small ncRNAs, such as microRNAs and tdRs, in mouse placenta/decidua, a unique interface for the communication between the foetus and the mother. They showed that specific tdRs are ubiquitously expressed in the placenta, but in response to maternal immune activation they change dynamically at the maternal-foetal interface, proposing the possibility that tdRs can play an active role in adaptation to environmental stress and developmental changes [26].

Finally, two review articles in this Special Focus discuss emerging roles of tdRs in distinct biological processes such as immunity and carcinogenesis [27] as well as mammalian development and stem cell maintenance [28]. They summarize biogenesis and functions of tdRs in these processes and discuss potential therapeutic implications about specific tdRs in the treatment of human diseases.

In conclusion, although plenty of questions still remain, we are entering an exciting time of tdR exploration. In the coming years, we will witness increasingly comprehensive insight how tdRs are integrated into complex gene regulatory networks and how they shape life, orchestrate cellular and organismal stress response and contribute to human health and disease.

Disclosure statement

No potential conflict of interest was reported by the authors.

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