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POINT OF VIEW

What is an RNA? *A top layer for RNA classification*

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ABSTRACT

Every ribonucleic acid begins its cellular life as a transcript. If the transcript or its processing product has a function it should be regarded an RNA. Nonfunctional transcripts, by-products from processing, degradation intermediates, even those originating from (functional) RNAs, and non-functional products of transcriptional gene regulation accomplished via the act of transcription, as well as stochastic (co) transcripts could simply be addressed as transcripts (class 0). The copious functional RNAs (class I), often maturing after one or more processing steps, already are systematized into ever expanding sub-classifications ranging from micro RNAs to rRNAs. Established sub-classifications addressing a wide functional diversity remain unaffected. mRNAs (class II) are distinct from any other RNA by virtue of their potential to be translated into (poly)peptide(s) on ribosomes. We are *not* proposing a novel RNA classification, but wish to add a basic concept with existing terminology (transcript, RNA, and mRNA) that should serve as an additional framework for carefully delineating RNA function from an avalanche of RNA sequencing data. At the same time, this top level hierarchical model should illuminate important principles of RNA evolution and biology thus heightening our awareness that in biology boundaries and categorizations are typically fuzzy.

Historical considerations

As RNomes continue to increase in complexity and with a new RNA category being proclaimed almost monthly,[1-3] it is time to contemplate the basic question as to what an RNA *is.* About a century ago, the veil on the structure of a substance initially known as yeast nucleic acid or zymonucleic acid, later as pentose nucleic acid (PNA), and finally as ribonucleic acid, began to lift largely due to the contributions of Phoebus A.T. Levene and Jean L.A. Brachet.[4-6] Since the late fifties to early sixties, we learned that most if not all cellular ribonucleic acid is copied from DNA templates in an enzymatic process.[7] Around the same time, knowledge concerning RNA's participation in protein biosynthesis emerged, including microsomal RNA (rRNA) and soluble RNA (tRNA) as well as mRNA.[8] In the sixties and seventies other RNAs were detected and characterized, including members of RNA families that we classify today as small nuclear and nucleolar RNAs.[9-11]. In those days, RNAs other than mRNAs were termed structural RNAs, presumably owing to their anticipated structural tasks, following rRNA that was simply regarded as a rack or backbone for the "only" functional components, the ribosomal proteins.[8] An additional motivation for this term might have been the presumption that these RNA species could form higher order structures (secondary and tertiary structures) akin to tRNA[12] - at that time in alleged contrast to mRNAs. Remarkably, in the early 60s, isolated investigators predicted the roles of RNA not only in the evolution of life but also as functional and regulatory entities in extant organisms.[13]

Apart from the few aforementioned RNAs as well as others thought to be fossils from the RNA world, the contributions of RNAs in cellular function and evolution were largely underappreciated. Only now, after 3-4 decades, does RNA finally receive the recognition it truly merits and in some instances, this attention has led to an exaggeration of its functions. All DNA-templated RNAs are transcripts, but the question is whether every transcript, albeit clearly a ribonucleic acid in the chemical sense, deserves to be classified as an *RNA*. In the biological context, we ought to be more discriminating.

The controversy

Recently, large-scale experimental approaches, such as microchip analysis and ultra-deep sequencing of the cellular RNA componentry, revealed copious transcripts including those of very low abundance. A debate is raging as to which fraction exerts a function. One camp claims that most if not all identified transcripts, including detectable degradation products, are functional.[14-20]. The other side stipulates that although genomes of most organisms likely encode, in addition to mRNAs, as many as thousands or tens of thousands of functional RNAs, a large fraction, in particular the low abundant transcripts, merely represent transcriptional noise resulting from stochastic initiation in intergenic regions,[20-27] previously un-annotated (alternatively spliced) untranslated regions (UTRs),[28] read-throughs of *bona fide* gene termination sites,[29] and more or less stable debris or leftovers from the processing

of primary/precursor transcripts. The most prominent are discarded introns, internal transcribed spacers, leaders, trailers, etc., but occasionally also sequences comprising spliced exons if, for example, the genes are merely hosts for miRNAs or snoRNAs and the corresponding exons lost (or never had) protein coding capacity.[30] as well Furthermore, molecules generated during the turnover and degradation of mature RNAs fall into this class.[31-37] Notably, transcriptional interference, which is gaining acceptance as yet another important layer of gene regulation, produces ribonucleic acids whose sequences are completely irrelevant; simply the act of transcription mediates an effect on, for example, a downstream promoter, including the blockade of transcription factor binding sites[38-41] or the alteration of chromatin structure.[42,43]

The difference between a transcript and an RNA

If we think about a ribonucleic acid chain as an RNA, it is usually with the association of a certain functional (including regulatory) or structural role – in analogy to a protein. This should not be expected from every stochastic transcript or degradation product or other "non-functional" ribonucleic acids. In keeping with the tradition of the oxymoronic term non-coding ribonucleic acids (ncRNAs, see also below) for functional non-mRNAs[c], one is tempted to address the ribonucleic acids that are devoid of function as "non-RNAs." Instead, we endorse the generic term *transcripts* for this class of ribonucleic acid, because some members of this category had previously been designated as stable untranslated transcripts or transcripts of unknown function (SUTs, TUFs).[46,47] As it is not trivial to assess whether such transcripts are never translated or otherwise functional,[48] and as ultra-deep RNA sequencing also identifies spurious or background transcripts of very low abundance, we prefer the simple term transcripts, adding that it includes stochastic transcripts, leftovers from primary transcript processing, or degradation products from the turnover of mature RNAs as well as products from regulatory acts of transcription. It follows then that transcripts constitute a further category, class 0[d], in addition to class I (functional including structural RNA)[e] and class II (mRNA encoding peptide or protein) RNAs.[49]

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[c] Possibly, the sole function of miRNAs is to act as guides complementary to regions of mRNA 3′UTRs (usually), placing repressive proteins for translational regulation or stability onto the targeted mRNAs. Likewise, snoRNAs act as guides complementary to RNAs (mostly rRNAs), exactly determining the nucleosides to be modified by enzymes bound to snoRNAs. These RNA classes clearly carry (anti)codes according to Barbieri43 and Trifonov44. and thus, should not be addressed as non-coding RNAs.

[d] Of course, any RNA begins life as a transcript and a few of the functional RNAs remain unprocessed. Thus the primary transcript constitutes the functional RNA, but it is the function that usually sets RNAs apart from nonfunctional transcripts or parts thereof: Many processed parts of primary transcripts (e.g., introns, leaders, trailers) are nonfunctional, perhaps performing a temporary function such as providing higher order structure, e.g., necessary for processing. Yet, these ribonucleic acids should not be regarded as RNAs in the considerations presented here, due to their lack of function (biology over chemistry). Usually, a transcript without being processed does not function despite the fact that it harbors functional RNAs (e.g., pre-mRNAs, pre-rRNA, pre-miRNAs, etc.).

[e] It does not matter how tiny (e.g., miRNA, piRNA), or large (e.g., Xist) an RNA is or whether an RNA was chemically synthesized. The latter is an RNA since most, if not all, molecules are being designed to exert a function, *in vivo* or *in vitro*. Viral RNA genomes are RNAs where one of the functions is the mobility from cell to cell and host to host.

Gray areas

A recurring theme in biology is the frequent lack of clear boundaries or states; we are confronted instead with broad, fuzzy interphases or with chimera (Fig. 1). Evidently, most mRNAs (class II) not only feature an open reading frame (ORF) but also contain other codes in UTRs or even within ORFs. Those codes function as regulatory elements influencing the stability or translatability of mRNAs, and thus, mRNAs are chimeras of class I and II RNAs.[50-53] The borders between RNA and mRNA are also fuzzy because some *bona fide* class I RNAs might also encode ORFs, which are occasionally translated[54] (i.e., they are bifunctional[f] RNAs).[56,57] Likewise, an RNA might be in the process of exaptation as mRNA.[58] An example for a chimera is the bacterial hybrid of a transfer and a mRNA, tmRNA, formerly known as 10Sa RNA, which functions to deblock ribosomes engaged in translating truncated mRNAs devoid of an in-frame stop codon.[59] Furthermore, rRNA also encodes short peptides conveying antibiotic resistance.[60]

Interphases between transcripts on one hand and RNAs or mRNAs on the other, are also of interest. A broad range is expected between transcripts and RNAs, with some transcripts on their way to exaptation and a significant fraction on their way to oblivion.[21,61-63] In contrast to the interphase between transcripts and RNAs, the range between transcripts and mRNA is presumably somewhat condensed by mRNA's requirement of at least temporal ribosome association, which can be assessed experimentally.[64] While this does not necessarily assure translatability, its absence would not clearly exclude it, as this association might not have been investigated in the appropriate cell types or developmental times.

Ribonucleic acids that serve as binding partners for one or more proteins to bring them into close proximity for a combined function or to shuttle them to a specific subcellular location exert a task and hence are RNAs (class I). Then again, any transcript or degradation product can become or remain decorated with protein, which does not automatically imply a function; hence, they would constitute transcripts (class 0). In contrast, ribonucleic acids that act as decoys or sinks for other RNAs, proteins, or other molecules should be categorized as RNAs (class I).[65-68] This includes some of the circular RNAs (circRNAs) and non-translatable transcripts generated from duplicated genes, such as retroposed pseudogenes.[69] However, the majority of circRNAs, often generated by aberrant splicing, is expected to be devoid of function and consequently should be considered transcripts (class 0).[70] Likewise, a ribonucleic acid generated by a regulatory act of transcription also is not an RNA in the biological sense. As argued above, this does not rule out a fortuitous future exaptation of any class 0 transcript as a functional RNA or mRNA.[21] Notably, a role for extra transcripts as evolutionary raw material was proposed by Henry Harris half a century ago.[71,72]

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[f] The term bifunctional RNA is also used for other molecules with two domains. For example, an antisense RNA sequence specific to a target hnRNA and an untethered RNA segment that serves as a binding platform for splicing factors to guide certain desired splice variants as potential therapeutic agents. ADDIN EN. CITE.DATA 55.
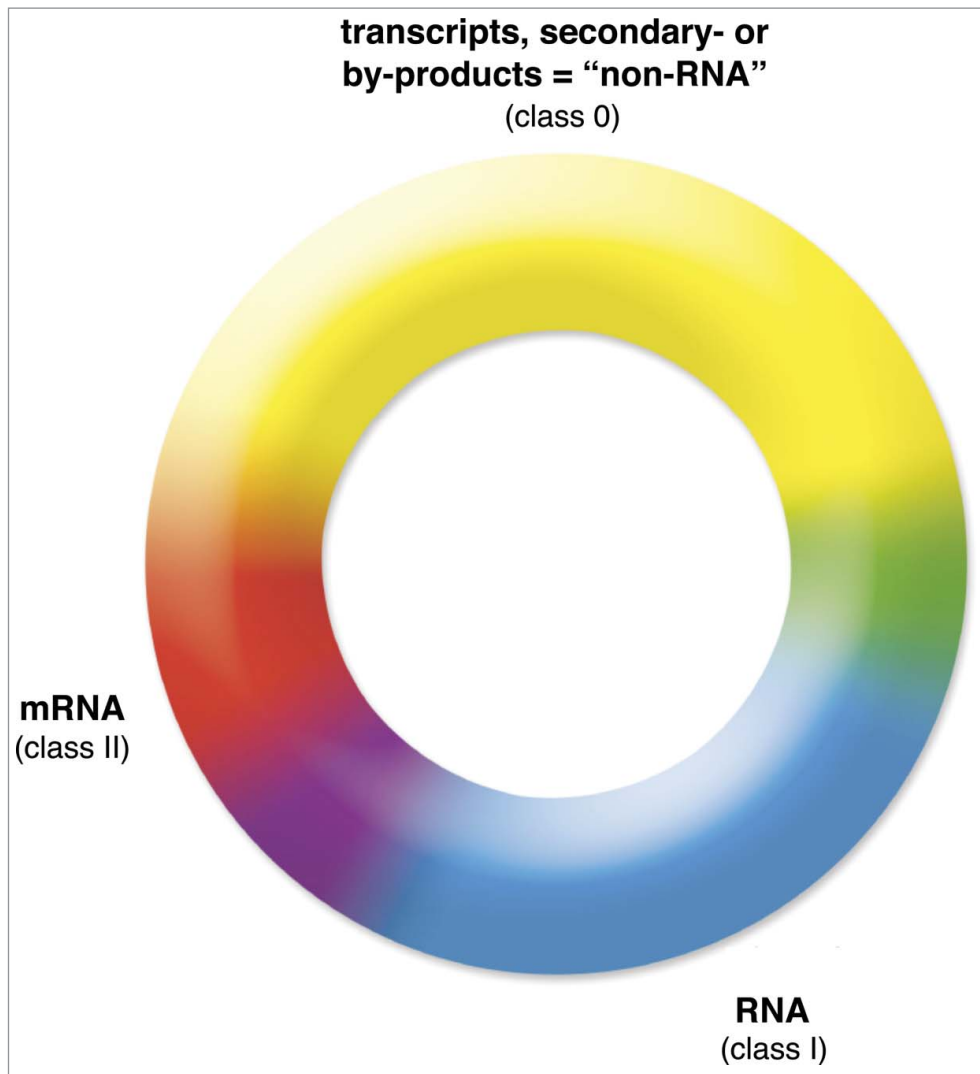
transcripts, secondary- or by-products = "non-RNA"
(class 0)

mRNA
(class II)

RNA
(class I)

Figure 1. A basic classification of transcripts and RNAs including the fuzziness of boundaries. Depicted is a continuum of the 3 ribonucleic acid superclasses: the first includes stochastic transcripts, other transcripts generated during gene regulation by acts of transcription, secondary products of RNA maturation such as introns etc., and RNA turnover products (*transcripts*, class 0) in yellow, functional RNAs (*RNAs*, class I) in blue, and classic mRNAs (*mRNAs*, class II) in red. Hybrid zones, interphases, or transitions between the 3 classes, for example, reflecting chimeras of 2 classes or those in the process of exaptation or abandonment are shown in purple, orange, and green. This figure does not represent quantitative measures of ribonucleic acid species or their abundances.

Concluding remarks

If we address a functional ribonucleic acid as RNA (class I), a translated or messenger ribonucleic acid as mRNA (class II) and everything else as a transcript (class 0), we do not need terms such as non-protein coding RNA (npcRNA), which sometimes might be subject to revision if a templated translation product is subsequently revealed. Neither do we need the unfortunate term non-coding RNA (ncRNA), because it reduces the RNA to something that it is not, obscures the fact that there is a gene coding for it ("a gene encoding a non-coding RNA"), and ignores the fact that RNAs carry many codes other than the one translated at ribosomes.[44,45] In any event, there is absolutely no need for the "nc" qualifier for RNA, as the term mRNA (mRNA) already provides the necessary qualifying differentiator. We do not propose to abandon clearly defined categories of RNAs, such as, for example, tRNAs, rRNAs, snRNAs, snoRNAs, miRNAs, or piRNAs, Instead, we simply add a top-level hierarchical layer to RNA classification using established categories of ribonucleic acids. Importantly, with this basic framework in mind, it should be easier to comprehend that defining a (sub)class of RNA does not necessarily imply that all its members are functional, such as any circular RNA by-product from splicing or any RNA snippet that happens to be in a size range of miRNAs or piRNAs.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

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