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The Old and New RNA World

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Abstract

Among the numerous hypotheses offering a scenario for the origin of life on Earth, the one called "The RNA World" has gained the most attention. According to this hypothesis RNA acted as a genetic information storage material, as a catalyst of all metabolic reactions, and as a regulator of all processes in the primordial world. Various experiments show that RNA molecules could have been synthesized abiotically, with the potential to mediate a whole repertoire of metabolic reactions. Ribozymes carrying out aminoacyl-tRNA reactions have been found in SELEX (systematic evolution of ligands by exponential enrichment) approaches and the development of a ribosome from a RNA-built protoribosome is easy to imagine. Transfer RNA aminoacylation, protoribosome origin, and the availability of amino acids on early Earth allowed the genetic code to evolve. Encoded proteins most likely stabilized RNA molecules and were able to create channels across membranes. In the modern cell, DNA replaced RNA as the main depositor of genetic information and proteins carry out almost all metabolic reactions. However, RNA is still playing versatile, crucial roles in the cell. Apart from its classical functions in the cell, a huge small RNA world is controlling gene expression, chromatin condensation, response to environmental cues, and protecting the cell against the invasion of various nucleic acids forms. Long non-coding RNAs act as crucial gene expression regulators. Riboswitches act at the level of transcription, splicing or translation and mediate feedback regulation on biosynthesis and transport of the ligand they sense. Alternative splicing generates genetic variability and increases the protein repertoire in response to developmental or environmental changes. All these regulatory functions are essential in shaping cell plasticity in the changing milieu. Recent discoveries of new, unexpected and important functions of RNA molecules support the hypothesis that we live in a New RNA World.

Keywords: RNA World hypothesis; ribozyme; small RNAs; long noncoding RNAs; riboswitches; alternative splicing; New RNA World

The origin of life

Among numerous hypotheses offering a scenario for the origin of life on Earth the one called "The RNA World" has gained the most attention. The break-through for this hypothesis, originally described independently by Leslie Orgel (working then at the Salk Institute for Biological Studies in San Diego) [1], Francis Crick (then at the Medical Research Council in England) [2], and Charles Woese (of the University of Illinois) [3] in the late 1960s of the XXth century suggested a world in which RNA catalyzed all the reactions necessary to create an ancestor of a living cell that was able to survive and replicate. In 1977, Manfred Eigen and Peter Schuster described the key features of a molecule with the potential to start life: ability to replicate, in other words to have molecular memory; to carry out all metabolic reactions, to be a catalyst; and to exhibit genetic variability, i.e. to be able to evolve [4]. The only key feature of the RNA molecules that raised problems those days as an originator of Life was the possibility to catalyze biochemical reactions. In 1983, Thomas R. Cech (University of Colorado at Boulder) and Sidney Altman (Yale University) independently discovered the first known enzymes made of RNA [5,6]. The term ribozyme was coined for RNA molecules that exhibit catalytic activities. These discoveries revealed that ancient RNA might have been catalytic and also a progenitor molecule that gave rise to life.

Abiotic synthesis of RNA components and RNA

If RNA molecules started life on Earth they must have been synthesized abiotically. RNA is built from nucleotides, which in turn are composed of a ribose, nitrogen base, and phosphate group(s). The experiments that were carried out during the last six decades show that nitrogen bases can be formed non-enzymatically in a range of experiments that simulate conditions scientists believe to be present on early

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Earth. However, for a long time there was no evidence that ribose molecule could be formed selectively. The addition of nucleobases to ribose is inefficient in the case of purines and does not occur at all in the case of pyrimidines. These difficulties have been recently circumvented through a non-enzymatic pathway in which arabinose amino-oxazline and anhydronucleoside intermediates play a key role leading to the synthesis of activated pyrimidine ribonucleotides. These intermediates are formed from plausible prebiotic feedstock molecules and phosphate. Phosphate, apart from its other functions in the pathway, is added to the ribonucleoside at the late stage of the synthesis [7,8]. In another course of experiments, an alternative, efficient and selective synthesis of pyrimidine ribonucleoside-2′,3′-cyclic phosphates has been demonstrated in conditions simulating the prebiotic environment of the early Earth [9].

Assuming the ribonucleotides could be formed on early Earth, there is still a need to show these molecules can polymerize and form RNA. Recently, it has been shown that the 2′-hydroxyl group of oligoribonucleotide-3′-phosphates can be chemoselectively acetylated in water under prebiotically credible conditions, which allows rapid and efficient template-directed 3′-5′ ligation. Moreover, the 2′-O-acetyl group at the ligation junctions of the product RNA strand can be removed under conditions that leave the newly formed 3′-5′ phosphodiester bonds intact. These results suggest a prebiotic pathway from ribonucleoside-2′,3′-cyclic phosphates to predominantly 3′,5′-linked RNA via partially 2′-O-acetylated RNA [10].

In addition, a recent paper by Adamala and Szostak provides strong evidence for nonenzymatic template-directed RNA synthesis inside model protocells representing fatty acid vesicles [10]. All these data concerning the possibility to abiotically generate ribonucleotides and to synthesize non-enzymatically template-directed RNA support the RNA World hypothesis.

RNA can promote various metabolic activities

Tom Cech and Sidney Altman provided the first evidence of RNA being a catalyst [5,6]. Since then naturally occurring ribozymes have been found in prokaryotes, eukaryotes, and viruses. Their functions can be grouped as follows: self-cleaving ribozymes [e.g. hammerhead, hairpin, HDV, VS ribozymes, autocatalytic RNA cleavage in the human β-globin pre-mRNA – so called CoTC element (co-transcriptional cleavage)], self-splicing ribozymes (e.g. self-splicing introns of group I and II, and GRI1 ribozyme), ribonucleases acting in trans (RNase P) and ribozymes displaying peptidyl-transferase activity (ribosome) [11].

However, the existence of the RNA World would require a much larger number of pathways and catalysts that would resemble modern metabolism. Invented over two decades ago, the experimental approach termed SELEX (systematic evolution of ligands by exponential enrichment) represents a powerful tool to select unnatural ribozymes selected in vitro. The overwhelming variety of RNA catalysts that were obtained with the help of the SELEX approach shed light on the potential of catalytic activities in the RNA World [12]. Ribozymes with the ligase activity that utilize a RNA with a 5′-triphosphate as a substrate and form 3′-5′ phosphodiester bonds at the ligation junctions have been isolated [13]. Moreover, the group of Bartel selected a ribozyme capable of extending a primer for more than one helical turn of RNA in a template-dependent polymerization reaction [14]. The activity of this ribozyme resembles the reaction carried out by the RNA dependent RNA polymerase and strengthens the possibility for the existence of a ribozyme acting as an RNA polymerase in the primordial, RNA World. A ribozyme able to conduct specific tRNA aminoacylation has been obtained supporting the idea of RNA-controlled protein synthesis in the RNA World [15]. Redox-ribozymes, resembling the activity of alcohol dehydrogenase have been isolated [16]. Secondary metabolites are thought to be synthesized via Diels–Alder reaction (e.g. enantiospecific carbon-carbon bond reaction). Diels–Alderase ribozyme has been obtained and its structure and mechanism of the reaction was solved [17].

Coenzymes represent small compounds that provide reactive groups for various protein enzymes. Many coenzymes contain ribonucleotide moieties suggesting the existence of coenzyme-binding RNAs in the RNA World and probably represent the present-day cell surviving vestiges of that time. Jadhav and Yarus managed to isolate in frames of the SELEX approach trans-capping ribozymes able to add coenzyme A (CoA) to the 5′ end of RNA containing only a short terminal specific sequence followed by a random internal sequence. From the trans-capped RNA pool they obtained a ribozyme able to transfer CoA via its SH group to biotinylated AMP. These ribozymes also produce crucial metabolic intermediates like acetyl-CoA and butyryl-CoA [18]. From this short overview it is shown that RNA molecules can offer a broad spectrum of catalytic activities necessary to support various metabolic pathways.

First RNA-encoded protein syntheses

Modern cell metabolism is based mainly on protein enzymes carrying out almost the entire repertoire of cellular catalytic reactions. Assuming the existence of the RNA World, how did proteins come into existence in the world controlled by RNA? Firstly, almost all amino acids were present in the primordial world as proved by the experiments simulating the conditions present on the early Earth [19]. Secondly, every step of biological peptide synthesis can be catalyzed by ribozymes [20]: (***i***) ribozymes can aminoacylate the 2′(3′) tRNA termini via 5′ aminoacyl-AMP; (***ii***) a small ribozyme can catalyze peptidyl-RNA synthesis [21], and (***iii***) peptide bond formation is observed in the ribosome, which is a ribozyme [22]. On the other hand, a protoribosome was probably built from RNA and evolved from a duplication of a ribozyme center able to bind aminoacyl-tRNA-like molecules. Having binding sites for two aminoacyl-tRNA-like molecules, one can speculate that the duplicated ribozyme center became capable of peptide bond formation. Assuming the specificity of ribozymes carrying out aminoacyl-tRNA reactions and the development of a ribosome one can easily imagine the origin of the genetic code.

Why proteins substituted RNA catalysts?

There are 20 amino acids that can be incorporated into the peptide chain during ribosomal protein synthesis in modern cells. These amino acids contain more chemical groups than the four nucleotides present in RNA molecules, and thereby offer a broader potential for different functions. Since the majority of amino acids were already present in the primordial soup, one cannot neglect their existence. What was the role of amino acids in the RNA World? One attractive possibility comes from the observations of the modern cell nucleotide de novo synthesis: purine rings are synthesized from aspartic acid, glutamine, and glycine, while the formation of a pyrimidine ring involves aspartic acid. Thus, amino acids could serve in the RNA World as substrates for the synthesis of RNA components. What could be the attractiveness of primitive first RNA-encoded peptides to the RNA world? A synthesis of the first peptides could not lead to the production of efficient catalysts. However, even short peptides composed of serine/leucine motifs are able to form ion-channels across membranes that allows the exchange of metabolites between the membrane-bound riboorganism and the environment [23]. The possibility to exchange the information between the proto-cell and the environment must have preceded the closure of RNA ribozyme metabolic centers within the fatty acid vesicles. Other attractive functions of simple peptides are the stabilization of RNA molecules. In a modern cell practically all RNA molecules form complexes with proteins, referred to as ribonucleoporteins (RNPs). RNase P, snRNPs, hnRNPs, snoRNPs, ribosomes, and telomerases are the best known examples of ribonucleoproteins in which proteins play structural and protective roles (RNase P, snRNP, snoRNP, hnRNP), structural, protective and catalytic roles (ribosome) and a catalytic role (telomerase). The role of proteins within RNPs mentioned above may reflect the evolutionary progress of protein functions. Finally enzymes that bind and use coenzymes as donors of reactive groups could have acted as ribonucleoproteins earlier in the course of evolution. In these RNPs, RNA was systematically shortened and coenzymes having only a ribonucleotide represent a final stage of RNA moiety atrophy.

DNA represents relatively young evolutionary achievement

Apart of several viruses genetic information is stored in DNA molecules. However, in the RNA World era, RNA can mediate both the storage of genetic material and catalytic activity. The comparison of DNA and RNA as genetic information storage material clearly shows DNA more suitable to fulfill these functions. DNA is a double stranded molecule in which antiparallel DNA strands contain complementary nucleotide sequences. Watson–Crick base pairing rule secures an additional control over the nucleotide sequence in both DNA strands. In addition, the lack of a 2′OH group in the deoxyribose renders DNA molecules more stable and resistant to chemical degradation. Thus DNA is more efficient for safe storage of genetic information. Are there any arguments supporting the relatively young age of DNA molecules? Firstly, in the modern cell deoxyribonucleotides are synthesized from ribonucleotides. Assuming RNA building blocks serve as substrates to synthesize deoxyribonucleotides one can speculate that ribonucleotides had to exist earlier than their deoxy- counterparts. Secondly, cellular metabolism uses ATP, CTP, UTP, and GTP but not their deoxy- counterparts in all biochemical reactions. This in turn suggests the existence of cellular metabolism long before DNA was created.

New RNA World

Is RNA playing a minor, secondary role in modern cell metabolism? Did proteins really replace RNA? The essential role of tRNAs, rRNAs, mRNAs, snRNAs, and snoRNAs in RNA metabolism or protein synthesis are known. Moreover, DNA replication is controlled by RNA in two ways: deoxyribonucleotides are made from ribonucleotides and main replicating enzymes, DNA-dependent DNA polymerases require an RNA primer to start DNA replication. Recent discoveries show that RNA molecules also play a crucial role in the regulation of gene expression (small RNAs and long non-coding RNAs, riboswitches) as well as in the boosting of cellular genetic diversity (alternative splicing). This chapter will focus on newly discovered roles of RNA molecules in plant cells.

Small RNA realm

The discovery of small RNAs (sRNAs) acting in the transcriptional and posttrancriptional gene silencing (PTGS) pathway was first reported by Hamilton and Baulcombe in plants and by Fire et al. in *Caenorhabditis elegans* [24,25]. Today there are two main classes of small RNAs operating in the plant cell: microRNAs and siRNAs (small interfering RNAs). The latter can be further divided into multiple categories of which the best-known examples are siRNAs (small interfering RNAs), ta-siRNAs (trans-acting siRNAs), ra-siRNA (repeat-associated siRNAs), and nat-siRNAs (natural-antisense siRNAs).

Small RNAs control gene expression at the posttranscriptional level

MicroRNAs

MicroRNAs represent a class of small RNAs of 18–24 nt in length that are encoded in eukaryotic genomes and posttranscriptionally downregulate the expression of target mRNAs. Plant miRNA genes (*MIRs*) are transcribed by RNA polymerase II and contain a cap structure at the 5′ end and polyA tail at the 3′ end [26]. A specific feature of primary microRNA transcripts is the formation of a stem and loop structure; within the stem microRNA and its partially complementary microRNA* are embedded. A duplex of microRNA and microRNA* is excised in a two-step reaction from the primary transcript by a protein complex referred

to as the microprocessor in which DCL1 (Dicer Like 1) enzyme plays a crucial role. miRNA : miRNA* is exported from the nucleus to the cytoplasm where the microRNA is incorporated into the RISC (RNA induced silencing complex) containing Argonaute 1 protein (Ago1, slicer) while miRNA* is in the majority of cases degraded. RISC complex guided by miRNA binds to a complementary mRNA and promotes mRNA cleavage (for review, see [27]). In some cases mRNA is not cleaved but its translation is inhibited [28]. The main targets of miRNAs are genes regulating key developmental processes such as flowering, flower, leaf and root morphogenesis (these are genes encoding mainly transcription factors) and providing plant response to stresses [stress-related genes, e.g. nucleotide binding site–leucine-rich repeat (NBS-LRR)]. MicroRNAs can be regarded as a rapid reaction force that enables fast developmental transitions and responses to various stresses.

Trans-acting siRNAs (ta-siRNAs)

Trans-acting siRNAs are a plant-specific class of siRNAs that were discovered several years ago [29]. Ta-siRNAs are produced from long non-coding RNAs that are derived from *TAS* loci. *TAS* loci-derived transcripts are initially cleaved by a microRNA the product of which is mainly recognized by RNA dependent RNA polymerase 6 (RdRP 6) and converted into the dsRNA. This class of dsRNA is recognized by DCL4 enzyme, which produces phased, 21 nt long ta-siRNAs proceeding in a step-wise manner along the dsRNA [30]. Unlike other siRNAs, ta-siRNAs silence the expression of genes that have little overall resemblance to the genes from which they originate. In this respect they resemble microRNAs. Similarly to microRNAs, ta-siRNAs downregulate posttranscriptionally cellular mRNAs by targeting RISC complex to a complementary mRNA. Trans-acting siRNAs are involved in the regulation of crucial plant developmental processes (e.g. the juvenile-to-adult transition in leaf development, lateral organ development) [31,32]. Increasing experimental evidence suggests that the originally triggered slicing of TAS transcripts leading to the ta-siRNA generation may induce a cascade of secondary siRNA production that targets other mRNAs [33,34]. Multiple steps in ta-siRNA-derived siRNA production enable the cell to precisely and quantitatively control metabolic processes. In this respect, the siRNA cascade resembles well-known cascades of kinases (e.g. MAP kinases) and transcription factors [35].

Small RNAs control plant response to abiotic stress

Natural antisense siRNAs (nat-siRNAs)

Nat-siRNAs (22–41 nt long) are produced from double stranded RNA regions formed by antisense transcripts derived from overlapping genes transcribed in opposite directions. They have been discovered in plants subjected to stress conditions. Generally one of the partially antisense transcripts is expressed constitutively while the other is stress-induced. dsRNA regions are cleaved by DCL2, DCL1/DCL4 thereby producing nat-siRNAs. Their production also requires RdRP6 activity, HYL1 (HYPONASTIC LEAVES 1) as well RNA Pol IV. Their biogenesis pathway is still not fully understood. It is known, that nat-siRNAs target the constitutively expressed mRNA [36–38]. Further studies on nat-siRNAs have revealed the existence of nat-siRNAs derived from partially antisense transcripts encoded in different parts of the genome [39]. Both types of nat-siRNAs are involved in the regulation of plant response to abiotic or biotic stresses.

Small RNAs control chromatin condensation

Repeat-associated siRNAs (ra-siRNAs)

Repeat-associated siRNAs [called also heterochromatic siRNAs (hc-siRNAs)] are a class of 23–24 nt long siRNAs generated from transposons, heterochromatic and repetitive genomic regions, representing the most abundant group of small RNAs in the plant cell [40,41]. They lead to de novo synthesis or maintain already existed DNA methylation of cognate genomic loci keeping them transcriptionally inactive. Biogenesis of ra-siRNA is nucleolar and requires the activity of atypical, plant specific RNA polmerases, RNA Pol IV and RNA Pol V. The requirement for the activity of RNA Pol II has also been shown in at least some cases [42]. RNA Pol IV is thought to transcribe methylated loci and transcripts are converted into double stranded RNA by RNA-dependent RNA polymerase 2 (RdRP 2). DsRNA is cleaved by DCL3 and ra-siRNAs associate with the Ago4 protein [43–45]. Ra-siRNA-guided Ago4 promotes DNA methylation in the cognate DNA regions leading to transposon, heterochromatin and repeat associated regional silencing. Surprisingly, ra-siRNAs can also move over long distances via phloem and guide DNA-target methylation in recipient cells [46]. Ra-siRNAs regulate gene expression via controlling chromatin condensation and control the accessibility of DNA to transcriptional machinery [47].

Small RNAs protect the eukaryotic cell against viral invasions

Short interfering RNAs (siRNAs)

Post-transcriptional gene silencing (i.e. RNAi in plants) represents mainly the viral RNA-targeting host innate immunity pathway. These siRNAs (also called viRNAs) represent a class of 21–24 nt long short RNAs that are produced mainly from duplexes of long RNA molecules having perfect complementarity. These can be found within the plant cell as a result of viral infection and viral RNA replication. dsRNA is recognized mainly by DCL2 and DCL4 family members which cleave the dsRNA producing short duplexes called siRNAs [48]. These are loaded into the RISC complex containing mainly Ago1, a family member of Argoanute proteins. One strand of the siRNA duplex is removed while the other one guides the RISC complex to execute posttranscriptional gene silencing. siRNAs can also be produced as a result of the activity of one of the cellular RNA-dependent RNA polymerases activity (RdRPs) which converts a single stranded RNA (ssRNA) into a double stranded RNA (dsRNA) in the case of transgenes, aberrant transcripts or single stranded long RNA containing hairpin structures (inverted repeats,

IR) expression. Primary siRNAs derived from viral- or transgene-dsRNA may also recruit RNA-dependent RNA polymerases to homologous ssRNA, and after dsRNA generation, to produce secondary siRNAs. This process leads to RNA silencing amplification. In plants, the silencing signal that requires the production of secondary siRNAs moves from cell-to-cell through plasmodestmata (short range signaling – up to 15 cells) or over long distances through the phloem (long-range silencing). Both processes require the activity of cellular RNA-dependent RNA polymerases [49]. The first one likely impacts virus movement while the other one helps immunizing the recipient tissues [50]. Taken together, viRNAs as well as transgene or IR-derived siRNAs, function to combat viral infection or to degrade transgene-derived or aberrant transcripts. Fig. 1 summarizes functions of different small RNA classes in the plant cell.

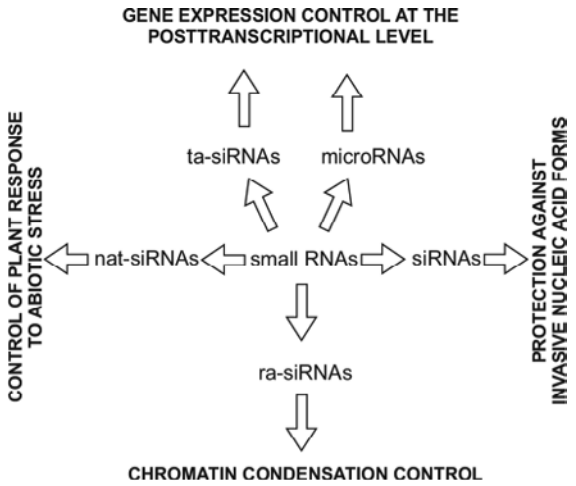

Fig. 1 Small RNA realm. A diagram showing different classes of plant sRNAs and their functions.

Long non-coding RNAs represent crucial regulators of gene expression

In recent years long intergenic non-coding RNAs (lincRNAs) have been identified as crucial regulators of gene expression. In *A. thaliana*, more than 2700 lincRNAs have been detected [51]. Plant lincRNAs are mainly transcribed by RNA Pol II and contain 5′ cap and polyA tail. Some lincRNAs are transcribed by RNA Pol IV and RNA Pol V and are connected with DNA methylation guided by 24 nt siRNAs produced from these transcripts [45]. Recently, Ariel et al have shown that auxin-driven transcription of lincRNA called APOLLO by RNA polymerases II and V controls the formation of a chromatin loop. Within this loop a promoter of a neighbor gene is included. Altering the expression of APOLLO regulates the chromatin loop formation. This control occurs via RNA-dependent DNA methylation, DNA demethylation, and Polycomb complexes. The dynamics of this loop is responsible for oscillatory gene expression pattern in response to developmental cues. This implies the existence of a general type of dynamic regulatory mechanism that enables rapid plant adaptation when exposed to environmental and developmental signals [52].

Riboswitches control cellular metabolism

Riboswitches represent metabolite-sensing domains of RNA [53,54]. They act at the level of transcription, splicing or translation and mediate feedback regulation on biosynthesis and transport of the ligand they sense. They are composed of an aptamer binding domain and a so-called expression platform. The first is responsible for metabolite binding while the other one executes gene expression regulation functions. The variety of ligands that riboswitches are able to bind includes metal ions, nucleobases, nucleosides, coenzymes, and amino acids. Riboswitches also sense physical parameters such as temperature [55]. More than 20 structurally different riboswitches have been identified so far [56]. The majority of riboswitches were found in prokaryotes, however there is one type discovered in filamentous fungi, green algae and land plants. This is a thiamin pyrophosphate (TPP)-binding riboswitch [57]. In all of these TPP-sensing riboswitches studied so far, the control of splicing reactions seems to play a key role in the gene regulatory mechanism. In land plants, the TPP riboswitch is found in the 3′UTR of thiamine metabolic genes. The best-studied example is the TPP riboswitch of *THIC* (*THIAMIN C*) gene. This gene encodes a key regulatory enzyme in the vitamin B1 (thiamin) biosynthesis. The riboswitch regulation involves splice site control by formation of mutually exclusive RNA structures. In the absence of TPP, pre-mRNA 3′UTR forms a structure in which access to the 5′ splice site for splicing machinery is blocked. These results in the presence of an unspliced 3′UTR in the mature *THIC* mRNA yielding stable mRNA and expression of THIC protein. However, in the elevated level of TPP *THIC* pre-mRNA binds this metabolite and 5′ slice site in the 3′UTR becomes accessible for splicing machinery and splicing takes place. As a result, a less-stable THIC mRNA is produced resulting in the downregulation of THIC protein levels (for review, see [58]). The plant TPP-sensing riboswitch as well as other riboswitch examples elucidated in prokaryotes show that a mechanism in which RNA structure is sensitive to an aptamer binding that results in the fine-tuning of cell metabolism. One can easily imagine the existence of such regulatory devices that allowed riboorganisms to sense and respond to environmental cues in the Old RNA World. Thus today operating riboswitches can be also regarded as putative descendants of RNA molecular regulators present already in the early forms of life.

Alternative splicing as a source of genetic variety

Alternative splicing (AS) generates multiple mRNA isoforms from a single primary transcript. There are several types of AS events: exon skipping/inclusion, intron retention, and alternative 5′/3′ splice site selection [59]. The AS mRNA isoforms pattern is regulated by tissue-specific or developmentally/environmentally induced expression of multiple SR proteins and hnRNPs that regulate quantitatively and qualitatively splicing events. In plants, over 60% of intron-containing pre-mRNAs undergo AS to produce a vast repertoire of mRNA isoforms [60]. In humans, more than 95% of protein mRNA coding genes undergo AS, generating

multiple mRNA isoforms and proteins [61]. Thus the value of over 60% of protein-coding transcripts undergoing AS events calculated for plants can still be underestimated since not all tissues, developmental stages and life conditions of plants have been studied in this respect. Is AS of functional importance? More and more studies indicate that the functionality of mRNA isoforms, and in many cases consequently isoproteins, is crucial for plant development and response to environmental changes. A transcript of *Arabidopsis ZINC-INDUCED FACILITATOR-LIKE 1* (*ZIFL1*) gene belonging to the major facilitator superfamily (MFS) transporter genes undergoes AS via alternative 3′ splice site selection in one of the multiple introns generating two mRNAs encoding longer and truncated protein forms. AS is tissue-specific and the full-length protein (ZIFL1.1) is detected in the tonoplasts of root cells while the truncated version (ZIFL1.3) – in the plasma membrane of leaf stomatal guard cells. ZIFL1.1 transporter regulates various root auxin-related processes, while ZIFL1.3 isoform mediates drought tolerance by regulating stomatal closure. This example shows that AS is responsible for tissue-specific expression of protein isoforms exhibiting different functions in plant development and response to environmental stimuli [62]. Another example shown in the work of the same group proved that intron retention in the 5′UTR of the novel zinc induced facilitator 2 (ZIF2) transporter enhances translation to promote zinc tolerance in *Arabidopsis*. Two mRNA isoforms with shorter (ZIF2.1, spliced version) or longer (ZIF2.2 intron retaining version) 5′UTR encoding the same protein are obtained. The higher amount of Zn in the soil induces the generation of a longer mRNA isoform, which confers greater zinc tolerance due to enhanced protein synthesis. Moreover, a stem and loop structure that is formed in the case of a longer 5′UTR immediately upstream of the start codon is largely responsible for the zinc-dependent efficient translation [63]. This example shows that AS may generate heavy metal-responsive mRNA isoforms with different susceptibility for translation. Recently published examples by the group of Paula Duque underscore the importance of alternative splicing in the regulatory network of a eukaryotic cell.

Conclusions

The Old and the New RNA Worlds seem to be metabolically connected. In the hypothetical RNA World, RNA molecules played roles of genomes, catalysts, and regulators. Some of these functions in modern cells have been overtaken by other types of molecules, which were most likely created by RNA in the Old RNA World: DNA maintains and protects genetic information, proteins are the main cell catalysts. However, RNA is still playing versatile and crucial roles in the cell. For example, RNA is significant in DNA and protein synthesis and in transcriptome and protein biodiversity. The newly discovered realm of small RNA molecules and riboswitches also show that RNA is a powerful tool in gene expression regulation and in shaping cell metabolism. Fig. 2 summarizes functions played by RNA in the Old and New RNA Worlds.

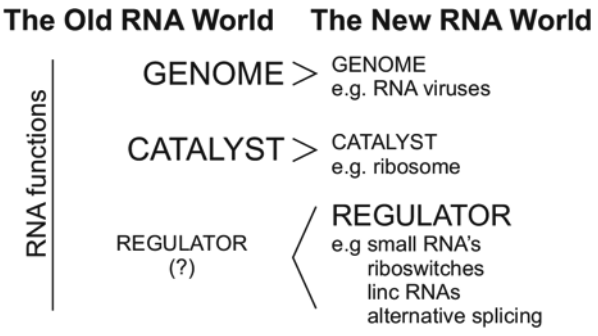

Fig. 2 A scheme presenting RNA functions in the Old RNA World and in the New RNA World.

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Competing interests

No competing interests have been declared.

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