Circular RNAs with hammerhead ribozymes encoded in eukaryotic genomes: The enemy at home

Marcos de la Peña and Amelia Cervera

Instituto de Biología Molecular y Celular de Plantas (Consejo Superior de Investigaciones Científicas-Universitat Politècnica de València) C/ Ingeniero Fausto Elio s/n, Valencia, Spain

ABSTRACT
A new family of non-autonomous retrotransposons with self-cleaving hammerhead ribozymes, the so-called retrozymes, has recently been found encoded in diverse plant genomes. These retroelements can be actively transcribed, and their RNAs accumulate in the cells as abundant non-coding circular RNAs (circRNAs) of small size (600–1000 nt). Related circRNAs with self-cleaving ribozymes had already been described in plants, and belong to a group of infectious RNA agents with an uncertain origin: the viroids and viroid-like satellites of plant RNA viruses. These pathogenic circRNAs show many structural similarities with retrozyome circRNAs, and both have been found to occur in flowering plants as heterogeneous RNA molecules of positive and negative polarities. Taking all these data together, we hypothesize that circRNAs encoded by genomic retrozymes could have given origin to infectious circRNAs with self-cleaving ribozymes. Moreover, we propose that retrozymes in time could have evolved from the ancient family of Penelope-like retroelements, which also harbour hammerhead ribozymes. Putative retrozyme sequences with hammerhead ribozymes have been detected as well in metazoan genomes, opening the door to a common occurrence of circRNAs with self-cleaving motifs among eukaryotes.

Introduction

With the discovery of catalytic RNAs or ribozymes more than 30 y ago,1,2 we started to become aware of the hidden capabilities of the RNA molecule in biology. Moreover, the ribozymes strongly supported the hypothesis of a prebiotic RNA world where the first living entities would have been based on RNA as both the genetic material and as catalyst.3-5 Only a few of those ancient ribozymes are believed to have remained in extant organisms, carrying out essential functions such as tRNA maturation by the RNase P,2 mRNA splicing by the spliceosome,6 and even protein translation by the ribosome.7 Among the simplest ribozymes described so far, there is an enigmatic family of small self-cleaving RNAs composed of 9 different classes: hammerhead (HHR),8,9 hairpin (HPR),10 human Hepatitis-δ (HDV),11 Varkud-satellite (VS),12 GlmS,13 twister,14 twister sister, hatchet and pistol15 ribozymes. The HHR was the first discovered and is one of the best-known members of this family. It is composed of a catalytic core of 15 conserved nucleotides surrounded by 3 double helices (I to III), which adopt a γ-shaped fold where helix I interacts with helix II through tertiary interactions required for efficient in vivo activity.16-18

There are 3 possible circularly permuted topologies for the HHR, named type-I, -II or -III, depending on the open-ended helix (Fig. 1). Originally described in the genomes of infectious circular RNAs (circRNAs) of plants, such as some viroids and viral satellite RNAs, the HHR catalyzes a self-cleaving transsterification that is required during the rolling-circle replication of these molecular replicons. A few HHRs were also exceptionally found in the DNA genomes of some unrelated eukaryotes,19-23 and mostly associated with repetitive sequences. In 2010, we reported the widespread occurrence of HHR motifs in genomes from bacteria to eukaryotes,24 including humans.25 These observations were confirmed and extended by other laboratories,26-28 revealing the HHR as a ubiquitous catalytic RNA motif in all life kingdoms.29 Other small self-cleaving RNAs, such as the HDV30 and twister ribozymes,14 have also been found widespread in DNA genomes, which corroborates that small catalytic RNAs are much more frequent than previously thought. Although the precise biologic roles of these genomic ribozymes are not well understood, a tight connection with mobile genetic elements such as retrotransposons has been reported in diverse eukaryotes.31-34 Retrotransposons are major components of most eukaryotic genomes, and can be classified in autonomous and non-autonomous. Autonomous retrotransposons encode the protein factors required for their own mobilization, which includes a retrotranscriptase (RT) responsible for...
for cDNA synthesis from an RNA transposition intermediate. Eukaryotic genomes can also contain many copies of small non-autonomous retroelements, which do not encode any protein and whose genomic mobility depends on the autonomous retrotransposons.35

Hammerhead ribozymes in plant genomes are part of a new family of non-autonomous retroelements: The retrozymes

We previously reported the presence in some plant genomes of HHR motifs, which in some cases occur as tandem repeats of a few hundred base pairs.24 More recent and deeper bioinformatic searches have extended these observations to the genomes of more than 40 plant species.34 Comparative genomic analysis revealed that the tandem HHR motifs were embedded within the sequence of what constitutes a novel family of non-autonomous retroelements, the retrozymes (retrotransposons with hammerhead ribozymes). These retroelements have sizes that range from around 1 to 1,5 kb, and show almost no sequence homology among distant plant genomes. All retrozymes, however, do display a similar structure: they are delimited by 4 bp target-site duplications (TSDs), with the HHRs embedded in direct long-terminal repeats (LTRs) of ~300–400 bp delimiting a unique central region (~300–600 bp), and flanked by the primer binding site or PBS (complementary to the tRNAMet sequence) and a poly-purine tract (PPT), both sequences required to prime DNA synthesis during the mobilization of LTR-retrotransposons36 (Fig. 2A, top). Retrozymes are similar to other non-autonomous retroelements of plants such as TRIMs37 and SMARTs38 (Fig. 2B) in that they rely on the machinery encoded by autonomous retrotransposons for their mobilization, most likely of the Ty3-gypsy family in the case of retrozymes.34 Plant retrozyme RNAs showed high self-cleaving activity in vitro, whereas northern blot hybridizations of RNAs from different plant tissues revealed abundant levels of circular and linear RNAs of the precise size encompassed by the HHRs,34 which is an indication of self-processing activity during in vivo transcription (Fig. 2A). Despite the lack of sequence identity between most retrozymes (with the exception of the short PPT, PBS and HHR motifs), secondary structure predictions for these circRNAs show a similar compact architecture with high stability (Fig. 3A), suggesting a selection pressure at this level. A final feature of retrozymes is their occurrence in vivo as heterogeneous RNA sequences of both the positive and negative polarities, which suggests that retrozyme circRNAs could be undergoing RNA-to-RNA replication through a rolling-circle mechanism, similar to the ones described for viroids and viral RNA satellites.34

The multiple connections between retrozyme and viroidal circRNAs

The first circRNAs reported in the literature were discovered in the 70s and called viroids.39,40 Based on different features, these small (240–470 nt) infectious non-protein coding circRNAs have been classified in 2 different families: Avsunviroidae and Pospiviroidae.41 Although a detailed description of the biology of these minimal plant pathogens can be found elsewhere,42 here we will summarize the major attributes of these 2 families. Members of the family Avsunviroidae (4 species, Table 1) are characterized by the presence of HHR motifs in both polarity strands and show no sequence similarity between them, whereas their RNA secondary structure can be either rod-like or highly branched (Fig. 3B) depending on the viroid size. On the other hand, members of the family Pospiviroidae (28 species) do not possess HHRs but several conserved sequence motifs, and show a rod-like secondary structure. In the 80s, a second group of infectious circRNAs with HHRs was found encapsidated in helper plant RNA viruses and called viroid-like satellite RNAs.43 The 9 known species of viroid-like satellites share many similarities with viroids (Table 1), such as being composed of a small (220–460 nt) circular RNA with a high degree of base pairing (Fig. 3C), replication through an analogous rolling-circle mechanism, and lack of protein-coding capacity (although an extraordinary case has been reported.
for in vivo suggests that both types of elements would require this enzyme (above 70%) of self-pairing (Fig. 3A). Moreover, the circRNAs have a stable branched secondary structure with a high degree slightly larger sizes (600–1000 nt), which are also predicted to circularize of the circRNA synthesis.34,45

looking for the origin of viroidal RNAs: Escaped introns, transposons or relics from the RNA world?

Since their discovery, infectious circRNAs have been considered as the lowest step of the biologic scale (so-called subviral agents) due to their minimal genome size and extreme simplicity as autonomously replicating entities. Regarding their possible origin, the first discovered viroids were proposed to be escaped spliceosomal introns as a result of some sequence similarity with snRNAs.46 The discovery and analysis of more viroid-like sequences led to propose a hypothetical origin from different retrotransposable elements based again on primary sequence similarities with either Ty-1 retrotransposons47 or Group I introns.48 With the landmark discovery of ribozymes as the most ancient biocatalysts, a latter hypothesis for the origin of the infectious circRNAs in the frontier of life posited that they might be “living fossils” from the primordial RNA world.59,60 Among the reasons supporting this idea are that the first entities of the RNA world should also have been small RNA replicons, most probably circular, with no protein-coding capacity but having simple self-processing ribozyme activities like the HHR.51 The monophyletic origin proposed for viroidal RNAs also lends support to the primordial origin hypothesis,52 although, due to the extremely small and fast-evolving genomes of these pathogens, such a common evolutionary origin should be regarded with caution.53 Nevertheless, the assumption of viroidal RNAs as survivors of the RNA world raises different questions difficult to explain,54 particularly, the identification of a reasonable evolutionary path accounting for the presence of these putative RNA fossils only in flowering plants (originated ~200 million years ago) but their absence in any ancestor of these plants, from algae to prokaryotes (4,100 million years ago). However, this direct connection between the RNA world and infectious circRNAs has been favored in the literature50,51,55,56 over the less fascinating hypothesis of escaped retroelements and/or introns. In this regard, the recent discovery of the widespread occurrence of small self-cleaving RNA motifs, such as the HHR or the HDV ribozyme, in viral, bacterial, archaeal, and eukaryotic genomes indicates that small ribozymes are not restricted to subviral RNA agents, such as viroidal RNAs with HHRs or the human Hepatitis-δ agent, but are very frequent components of DNA genomes. Furthermore, the discovery of a new family of retroelements with HHRs that spread through circRNAs, precisely in the genomes of flowering plants, as well as their structural similarities with infectious circRNAs, opens a more likely scenario where viral satellites and viroids with HHRs may have emerged de novo from the population of abundant retrozyme circRNAs present in plant transcriptomes. In this respect, it seems plausible that the circRNAs encoded by genomic retrozymes may be encapsidated by RNA viruses during plant infection in a similar way as reported for other host RNAs derived from Ty3-gypsy retroelements.57 Viral encapsidation of a retrozyme circRNA would be the first step in the biogenesis of a viroid-like satellite RNA, which would subsequently require the acquisition of recognition signals for the viral RNA polymerase to be replicated and, eventually, a second ribozyme in the opposite polarity (Table 1). On the other hand, de novo appearance of a HHR viroid from retrozyme circRNAs seems also feasible and would entail the acquisition of recognition motifs for plant RNA polymerases (a signal that may be already present, see before and34) and cell-to-cell movement, as well as the appearance of a second HHR in
the negative polarity. In this regard, the architecture of retrozyme circRNAs showing a highly self-paired HHR already offers a quasi-HHR sequence in the opposite polarity (Fig. 3). Viroidal RNAs with HHRs would not be the first infectious agents for which a cellular origin is suspected. The discovery of a novel class of cellular RT genes present in all major taxonomic groups but absent from selfish elements indicated that retrovirus evolved from genomic LTR retrotransposons rather than in the other way around. In view of all these data, an in planta origin for viroidal RNAs with HHRs would seem to us as a more realistic hypothesis than being ancient relics of precellular evolution.

**Discussion**

As recently reported, genomic retrozymes with HHRs show a patchy distribution among flowering plants, occurring numerously in different species, but being absent in some others. “Canonical” retrozymes (which contain HHRs, PBS and PPT motifs) seem to be mostly restricted to dicots, although the presence of putative retrozyme sequences with the characteristic tandem HHR copies have also been detected in primitive land plants (such as the spikemoss *Selaginella moellendorfii*), algae (such as *Chlamydomonas reinhardtii*), and even protists (such as oomycetes), suggesting that genome-encoded circRNAs with HHRs could occur in eukaryotes other than angiosperms. This scenario allows to propose a much simpler evolutionary path for small infectious circRNAs with HHRs of plants, which may have come by chance from the abundant reservoirs of retrozyme circRNAs present in plant transcriptomes. An obvious counter-argument is that retrozymes themselves could have originated from viroidal RNAs. Although this possibility cannot be ruled out, a better answer to this question can be found in the ancient family of the Penelope-like elements or PLEs. PLEs are a large family of retrotransposons found in many eukaryotes (including diatoms, algae and primitive land plants such as *Selaginellas*), which show phylogenetic connections with prokaryotic self-splicing introns and are believed to predate telomerases and most eukaryotic retrotransposons. Interestingly, PLEs show in their LTRs the

![Figure 3. Minimum free energy secondary structure predictions for (A) a retrozyme circRNA of *Jatropha curcas* (Entry KX273075.1), (B) the avsunviroid PLMVd (Entry M83545.1) and (C) the Nepovirus satellite RNA sTRSV (Entry M14879.1). HHR sequences are shown in purple (positive polarity) and green (negative polarity), and the PBS and PPT motifs of the retrozyme are shown in orange and blue, respectively. The corresponding structures of the HHRs motifs are shown under each circRNA structure and, with the exception of PLMVd HHRs, dotted lines indicate putative tertiary interactions between HHR loops. Self-cleavage sites are indicated with arrows. Kissing-loop interaction of PLMVd is also shown. Numbering for each circRNA starts at the self-cleavage site of the positive polarity HHR.](image)
Table 1. Compilation of circRNAs with hammerhead ribozymes from sequence databases.

| circRNAs with HHRs                      | Size (nt) | ~%GC | Ribozymes |
|-----------------------------------------|-----------|------|-----------|
| **Retozymes**                           |           |      |           |
| Fragaria ananassa retrozymes            | 673–?701  | 60   | 1 HHR     |
| Jatropha curcas retrozymes              | 693–790   | 57   | 1 HHR     |
| Citrus clementina retrozymes            | 663–602   | 56   | 1 HHR     |
| Eucalyptus camaldulensis retrozymes     | 900–1034  | 53   | 1 HHR     |
| **Viral satellite RNAs**                |           |      |           |
| Satellite of Rice Yellow Mottle Virus   | 220       | 64   | 1 HHR     |
| Satellite of Tobacco Mottle Virus       | 366       | 56   | 1 HHR     |
| Satellite of Solanum Nodiflorum Mottle | 377       | 56   | 1 HHR     |
| Virus (sSNMV)                           | 332–388   | 52   | 1 HHR     |
| Satellite of Subterranean Clover Mottle | 322–324   | 57   | 2 HHR     |
| Virus (sSCMoV)                          | 322       | 50   | 2 HHR     |
| Satellite of Cereal Yellow Dwarf Virus  | 359       | 55   | 1 HHR, 1 HPR |
| Satellite of Tobacco Ringspot Virus     | 457       | 54   | 1 HHR, 1 HPR |
| Satellite of Chicory Yellow Mottle Virus| 300–301   | 53   | 1 HHR, 1 HPR |
| **Viroids**                             |           |      |           |
| Chrysanthemum Chlorotic Mottle Viroid   | 396–401   | 56   | 2 HHR     |
| (CChMVd)                                |           |      |           |
| Peach Latent Mosaic Viroid (PLMVd)      | 321–354   | 53   | 2 HHR     |
| Eggplant Latent Viroid (ELVd)           | 331–335   | 52   | 2 HHR     |
| Avocado Sunblotch Viroid (ASBvd)        | 239–251   | 40   | 2 HHR     |
| **Other viroid-like circRNAs with HHRs**|           |      |           |
| Grapevine Hammerhead Viroid-like (GHvd)** | 375     | 57   | 2 HHR     |
| Apple Hammerhead Viroid-like (AHVd)**   | 434       | 52   | 2 HHR     |
| Carnation Small Viroid-like RNA/DNA     | 275       | 51   | 2 HHR     |
| (CarSY RNA/DNA)                         |           |      |           |
| Cherry Small Circular Viroid-like RNA   | 372–451   | 46   | 2 HHR     |
| (cssRNA)                                |           |      |           |
| Mulberry Small Circular Viroid-like     | 356–357   | 51   | 1 HHR, 1 HPR |
| (Mmd-v RNA)**                           |           |      |           |

HHR: Hammerhead ribozyme; HPR: Hairpin ribozyme

*More than 40 different plant genomes have been found to contain putative genomic retrozymes. Only the 4 examples experimentally shown to accumulate circRNAs in vivo are indicated.**

**Tentative viroids recently described through deep sequencing approaches.

***A circRNA described previously that was found to encode a HHR and a HPR (Contreras and De la Peña, unpublished results).

Concluding remarks

It has been long known that circular DNAs are common molecules in the biosphere, from prokaryotic plasmids to the genomes of most bacteriophages, bacteria, archaea and plastids. Circular RNAs, however, have been regarded as rare in biology until the recent confirmation that numerous life forms express stable circRNAs of different origins. Among them, it is noteworthy the finding of a myriad of splicing-derived circRNAs in eukaryotes with potential functions in transcription, splicing, or the biogenesis of small RNAs (for a review see62). In this regard, genome-encoded circRNAs with self-cleaving ribozymes represent a new level of complexity, whose study will offer functional clues as well as biotechnological applications in the fast-growing field of circular RNA molecules.

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ORCID

Marcos de la Peña http://orcid.org/0000-0002-7949-8459
Amelia Cervera http://orcid.org/0000-0001-6025-8773

References

1. Kruger K, Grabowski PJ, Zaug AJ, Sands J, Gottschling DE, Cech TR. Self-splicing RNA: Autoexcision and autocyclization of the ribosomal RNA intervening sequence of Tetrahymena. Cell 1982; 31:147-57; PMID:6297745; https://doi.org/10.1016/0022-2836(82)90014-7
2. Guerrier-Takada C, Gardiner K, Marsh T, Pace N, Altman S. The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. Cell 1983; 35:849-57; PMID:6197186; https://doi.org/10.1016/0022-2836(83)90117-4
3. Crick FH. The origin of the genetic code. J Mol Biol 1968; 38:367-79; PMID:9316887; https://doi.org/10.1016/0022-2836(68)90392-6
4. Orgel LE. Evolution of the genetic apparatus. J Mol Biol 1968; 38:93-103; PMID:5718557; https://doi.org/10.1016/0022-2836(68)90393-8
5. Woese CR. The fundamental nature of the genetic code: Prebiotic interactions between polynucleotides and polyanionic acids or their derivatives. Proc Natl Acad Sci U S A 1968; 59:110-7; PMID:5242115; https://doi.org/10.1073/pnas.59.1.110
6. Fica SM, Tuttle N, Novak T, Li NS, Lu J, Koobathingal P, Dai Q, Staley JP, Piccirilli JA. RNA catalyses nuclear pre-mRNA splicing. Cell 1983; 35:849-57; PMID:6197186; https://doi.org/10.1016/0022-2836(83)90117-4
7. Nissen P, Hansen J, Ban N, Moore PB, Steitz TA. The structural basis of ribosome activity in peptide bond synthesis. Science 2000; 289:920-30; PMID:10937990; https://doi.org/10.1126/science.289.5481.920
24. De la Pea, Martick M, Horan LH, Noller HF, Scott WG. A discontinuous hammerhead ribozyme. Nature 1986; 323:334-53; https://doi.org/10.1038/323334a0

25. De la Pea, Martick M, Gago S, Flores R. Peripheral regions of natural hammerhead ribozymes greatly increase their self-cleave activity. EMBO J 2003; 22:5631-70; PMID:14532128; https://doi.org/10.1093/emboj/cdg530

26. Martick M, Horan LH, Noller HF, Scott WG. A discontinuous hammerhead ribozyme embedded in a mammalian messenger RNA. Nature 2008; 454:899-902; PMID:18615019; https://doi.org/10.1038/nature07117

27. Perreault J, Weinberg Z, Roth A, Popescu O, Chartrand P, Ferbeyre G, Breaker RR. Identification of hammerhead ribozymes in all domains of life reveals novel structural variations. PLoS Comput Biol 2011; 7:e1002031; PMID:21573207; https://doi.org/10.1371/journal.pcbi.1002031

28. Sechafer C, Kalweit A, Steger G, Gräf S, Hammann C. From alpaca to zebrafish: Hammerhead ribozymes wherever you look. RNA 2011; 17:21-6; PMID:21081661; https://doi.org/10.1016/j.rna.2429911

29. Hammann C, Luptak A, Perreault J, de la Pea M. The ubiquitous hammerhead ribozyme. RNA 2012; 18:871-85; PMID:22454536; https://doi.org/10.1016/j.rna.2011.0314011.111

30. Webb CH, Riccitelli NJ, Rumiński DJ, Luptak A. Widespread occurrence of self-cleaving ribozymes. Science 2009; 326:953-5; PMID:19965505; https://doi.org/10.1126/science.1178084

31. Cervera A, De la Pea M. Eukaryotic penelope-like retroelements encode hammerhead ribozyme motifs. Mol Biol Evol 2014; 31:2941-7; PMID:25135949; https://doi.org/10.1093/molbev/mzu223

32. Eickbush DG, Eickbush TH. R2 retrotransposons encode a self-cleave- ing ribozyme for processing from an RNA cotranscript. Mol Cell Biol 2010; 30:3142-50; PMID:20421411; https://doi.org/10.1128/MCB.00300-10

33. Riccitelli NJ, Luptak A, Hume-Kropp C, Rumiński DJ, Luptak A. Processing and translation initiation of non-long terminal repeat retrotransposons by hepatitis delta virus (HDV)-like self-cleaving ribozymes. J Biol Chem 2011; 286:41286-95; PMID:21994949; https://doi.org/10.1074/jbc.M111.297283

34. Cervera A, Urbina D, de la Pea M. Retrozymes are a unique family of non-autonomous retrotransposons with hammerhead ribozymes that propagate in plants through circular RNAs. Genome Biol 2016; 17:135; PMID:27339130; https://doi.org/10.1186/s13059-016-0004-2

35. Wickner T, Sabot F, Hua-Van A, Bennetzen JL, Capy P, Chalhoub B, Flavell A, Leroy P, Morgante M, Panaud O, et al. A unified classification system for eukaryotic transposable elements. Nat Rev Genet 2007; 8:973-82; PMID:17984973; https://doi.org/10.1038/nrg2165

36. Gorinsky B, Gubensek F, Korids D. Evolutionary genomics of chro-moviruses in eukaroytes. Mol Biol Evol 2004; 21:781-98; PMID:14739248; https://doi.org/10.1093/molbev/msh057

37. Witte CP, Le QH, Bureau T, Kumar A. Terminal-repeat retrotransposons in miniature (TRIM) are involved in restructuring plant genomes. Proc Natl Acad Sci U S A 2001; 98:13778-83; PMID:11717436; https://doi.org/10.1073/pnas.241341898

38. Gao D, Chen J, Chen M, Meyers BC, Jackson S. A highly conserved, smallLTR retrotransposon that preferentially targets genes in grass genomes. PLoS One 2012; 7:e32010; PMID:22359654; https://doi.org/10.1371/journal.pone.0032010

39. Diener TO. Potato spindle tuber virus. IV. A replicating, low molecu-lar weight RNA. Virology 1971; 45:411-28; PMID:5095900; https://doi.org/10.1016/0042-6822(71)90342-4

40. Sanger HL, Klotz G, Riesner D, Gross HJ, Vidalakis G, Bureau T, Vladalak G, Owens RA. Current status of viroid taxonomy. Arch Virol 2014; 159:3467-78; PMID:25216773; https://doi.org/10.1007/s00705-014-2200-6

41. Flores R, Minoia S, Carbonell A, Gisel A, Delgado S, Lopez-Carrasco A, Navarro B, Di Serio F. Viroids, the simplest RNA replicons: How they manipulate their hosts for being propagated and how their hosts react for containing the infection. Virus Res 2015; 209:136-45; PMID:25735852; https://doi.org/10.1016/j.virusres.2015.02.027

42. Frohlich N, Davies C, Hatta T, Gould AR, Francki RI. Studies on encapsidated viroid-like RNA I. Characterization of velvet tobacco mottle virus. Virology 1981; 108:111-22; PMID:18635027; https://doi.org/10.1016/0042-6822(81)90531-6

43. Abou-Haidar MG, Venkataraman S, Golshani A, Liu B, Ahmad T. Novel coding, translation, and gene expression of a replicating covalently closed
circular RNA of 220 nt. Proc Natl Acad Sci U S A 2014; 111:14542-7; PMID:25253891; https://doi.org/10.1073/pnas.1402814111

45. Nohales MA, Molina-Serrano D, Flores R, Daros JA. Involvement of the chloroplastic isoform of tRNA ligase in the replication of viroids belonging to the family avsunviroidae. J Virol 2012; 86:8269-76; PMID:22623792; https://doi.org/10.1128/JVI.00629-12

46. Diener TO. Are viroids escaped introns? Proc Natl Acad Sci U S A 1981; 78:5014-5; PMID:16593072; https://doi.org/10.1073/pnas.78.8.5014

47. Kiefer MC, Owens RA, Diener TO. Structural similarities between viroids and transposable genetic elements. Proc Natl Acad Sci U S A 1983; 80:6234-8; PMID:6312450; https://doi.org/10.1073/pnas.80.20.6234

48. Dinter-Gottlieb G. Viroids and virusoids are related to group I introns. Proc Natl Acad Sci U S A 1986; 83:6250-4; PMID:3462692; https://doi.org/10.1073/pnas.83.17.6250

49. Diener TO. Circular RNAs: Relics of precellular evolution? Proc Natl Acad Sci U S A 1989; 86:9370-4; PMID:2480600; https://doi.org/10.1073/pnas.86.23.9370

50. Diener TO. Viroids: "living fossils" of primordial RNAs? Biol Direct 2016; 11:15; PMID:27016066; https://doi.org/10.1186/s13062-016-0116-7

51. Ma W, Yu C, Zhang W. Circularity and self-cleavage as a strategy for the emergence of a chromosome in the RNA-based protocol. Biol Direct 2013; 8:21; PMID:23971788; https://doi.org/10.1186/1745-6150-8-21

52. Elena SF, Dopazo J, de la Peña M, Flores R, Diener TO, Moya A. Phylogenetic analysis of viroid and viroid-like satellite RNAs from plants: A reassessment. J Mol Evol 2001; 53:155-9; PMID:11479686; https://doi.org/10.1007/s002390010203

53. Jenkins GM, Woelk CH, Rambaut A, Holmes EC. Testing the extent of sequence similarity among viroids, satellite RNAs, and hepatitis delta virus. J Mol Evol 2000; 50:98-102; PMID:10654264; https://doi.org/10.1007/s002399100011

54. Chela-Flores J. Are Viroids Molecular Fossils of the RNA World? J Theor Biol 1994; 166:163-6.

55. Bussiere F, Lafontaine D, Cote F, Beaudy D, Perreault JP. Evidence for a model ancestral viroid. Nucleic Acids Symp Ser 1995:143-4; PMID:8643352

56. Flores R, Gago-Zachert S, Serra P, Sanjuan R, Elena SF. Viroids: Survivors from the RNA world? Annu Rev microbiol 2014; 68:395-414; PMID:25002087; https://doi.org/10.1146/annurev-micro-091313-103416

57. Ghoshal K, Theilmann J, Reade R, Maghodia A, Rochon D. Encapsulation of Host RNAs by cucumber necrosis virus coat protein during both agroinfiltration and infection. J Virol 2015; 89:10748-61; PMID:26269190; https://doi.org/10.1128/JVI.01466-15

58. Gladyshev EA, Arkhipova IR. A widespread class of reverse transcriptase-related cellular genes. Proc Natl Acad Sci U S A 2011; 108:20311-6; PMID:21876125; https://doi.org/10.1073/pnas.1100266108

59. Koonin EV, Dolja VV. A virocentric perspective on the evolution of life. Curr Opin Virol 2013; 3:546-57; PMID:23850169; https://doi.org/10.1016/j.coovi.2013.06.008

60. Evgen’ev MB, Zelentsova H, Shostak N, Kozitsina M, Barskyi V, Lankenau DH, Corces VG. Penelope, a new family of transposable elements and its possible role in hybrid dysgenesis in Drosophila virilis. Proc Natl Acad Sci U S A 1997; 94:196-201; PMID:8990185; https://doi.org/10.1073/pnas.94.1.196

61. Gladyshev EA, Arkhipova IR. Telomere-associated endonuclease-deficient penelope-like retroelements in diverse eukaryotes. Proc Natl Acad Sci U S A 2007; 104:9352-7; PMID:17483479; https://doi.org/10.1073/pnas.0702471104

62. Barrett SP, Salzman J. Circular RNAs: Analysis, expression and potential functions. Development 2016; 143:1838-47; PMID:27246710; https://doi.org/10.1242/dev.128074

63. Wu Q, Wang Y, Cao M, Pantaleo V, Burgyan J, Li WX, Ding SW. Homology-independent discovery of replicating pathogenic circular RNAs by deep sequencing and a new computational algorithm. Proc Natl Acad Sci U S A 2012; 109:3938-43; PMID:22345560; https://doi.org/10.1073/pnas.1117815109

64. Zhang Z, Qi S, Tang N, Zhang X, Chen S, Zhu P, Ma L, Cheng J, Xu Y, Lu M, et al. Discovery of replicating circular RNAs by RNA-seq and computational algorithms. PLoS Pathog 2014; 10:e1004553; PMID:2503469; https://doi.org/10.1371/journal.ppat.1004553

65. Wang WB, Fei JM, Wu Y, Bai XC, Yu F, Shi GF, Li YF, Kuai YZ. A new report of a mosaic dwarf viroid-like disease on mulberry trees in China. Pol J Microbiol 2010; 59:33-6; PMID:20568527