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Enigmatic Entities of the Acellular World: Viruses, Viroids, and Virusoids

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ABSTRACT

Genetic material confined within the lipid based cellular boundaries was earlier considered synonymous with life. However, with the discovery of viruses in late 19th century, the existence of acellular biological entities was established. Viruses, viroids, and virusoids are unique entities which have different relationships with different life forms ranging from mutualistic to parasitic ones. These entities provide evidence in support of the idea of 'RNA world' in the origin of life on Earth. In the present time, viruses are relatively well studied but the same cannot be said for viroids and virusoids. There has been a growing focus on the impact of these entities, in terms of human welfare as well as their impact on susceptible varieties of plants. As a result, studying their origin, evolution and pathogenicity has become a subject of the uttermost importance. In this review, we have discussed different facets of viruses, viroids and virusoids like their historical background, classification and mode of entry and replication in the host. We have also summarized various possible theories on their origin and evolution and have provided our take on it. This work indicates the possibility that different viruses originated distinctly by utilizing different strategies and evolved further. Clues like small size and high GC content in genomes indicate that viroids must be an important component of the pre-cellular world and it is possible that they might have originated before viruses. Furthermore, as viroids and virusoids show certain conserved properties, it suggests a probable link between them.

Keywords: Viruses, Viroids, Virusoids, Origin, Evolution, Infection

INTRODUCTION

Viruses are a type of mobile genetic element (MGE) that consist of a genome enclosed by a protein capsid with some of them having an external lipid envelope (1,2). Viruses encode at least one protein which is a major component of the virion (1). Viroids are small (approx. 250-400 nucleotides), non-coding, non-translatable, non-encapsidated, single-stranded, circular RNAs that replicate by rolling circle mechanism utilizing the host enzymes (1,3-5) whereas virusoids are small RNAs (with a circular genome of 400 nucleotides or less) which depend upon helper viruses (HV) for their replication, encapsidation and transmission (6-8). Virusoid genomes do not generally code for proteins but a virusoid associated with the rice yellow mottle virus is known to code for a 16 kDa, highly basic protein (9).

Virusoids come under a larger group of satellite RNAs (satRNAs). SatRNAs and satellite viruses are associated with several viruses. SatRNAs are small RNA molecules (up to 1500 nucleotides in length), which depend on a HV to replicate, encapsidate and to transmit but rarely have any nucleotide sequence homology with HV (6). Satellite viruses, on the other hand, encode their own capsid protein and are not dependent on a HV, at least for encapsidation. For

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example, the satellite tobacco necrosis virus encodes its icosahedral capsid that accommodates its 1260 nucleotide long RNA, however, it depends upon its HV i.e., tobacco necrosis virus, for RNA polymerase which replicates the genomes of both the entities (10).

SatRNAs and satellite viruses are small molecular parasites. SatRNAs are mostly parasitic, but commensal or beneficial associations are also reported (6). SatRNAs have been subdivided into three subgroups (7), namely Small linear satRNAs (satRNA of less than 700 nucleotides), Large linear satRNAs (satRNA of 0.7-1.5 kb encoding a minimum of one non-structural protein) and Small circular satRNAs or Virusoids (circular RNA shorter than 400 nucleotides). In this work, only the third sub-group of satRNAs, i.e., small circular satRNAs, which is traditionally labelled as virusoids, is discussed. Viruses, viroids, and satellites (along with viriforms) are selfish MGEs which move between different hosts and can change their integration sites in the genomes of hosts. The relationship between hosts and MGEs vary from mutualism to parasitism (1).

Since the late 19th century, the world of acellular biological entities has been extensively studied. The discovery of the tobacco mosaic virus (TMV) provided a foundation for future research on other viruses as well as other acellular entities like viroids and virusoids. In the field of molecular biology, initially, the widely accepted idea was the "Central Dogma" which believed in unidirectional flow of genetic information from DNA to RNA to protein. This flow was presumed to be irreversible (11). However, with the discovery of viruses, the existence of acellular organisms was established, a fact which was further strengthened by subsequent discoveries of viroids and virusoids in the 20th century. These discoveries also challenged the widely accepted Central dogma, as certain viruses have RNA as their genome, which is reverse transcribed to produce DNA (12), whereas viroids and virusoids lack DNA altogether.

These acellular units are the smallest known biological entities. While the prevalence of viral infections is considerably high, the same cannot be said about viroids and virusoids. The latter two are known to infect plants. It is interesting to note that the very existence of these acellular entities supports the possibility of the 'RNA world'. This theory was proposed in 1986 and advocated for a living system which was entirely composed of RNA in the early evolutionary stages of these living systems (13). The existence of viroids and virusoids as RNAs and the use of RNA by some viruses as their genetic material hint towards a plausible RNA world stage in the initial stages of the origin of life on Earth (14).

In this review, we discuss some of the historical milestones achieved since the discovery of these acellular entities with a description of the different mechanisms by which these entities infect and replicate. We also elaborate upon the various theories which have been proposed for their origin and possible evolution in nature.

HISTORICAL BACKGROUND

Viruses

Viruses were first discovered around the end of the 19th century as small entities which were initially identified as unusual pathogens capable of passing through filters which bacteria couldn't cross (15). The first virus, TMV, was discovered in 1892 as a plant pathogen (16). In 1898, the foot-and-mouth disease virus (FMDV) was the second virus to be discovered which is now known to infect farm animals (17). It was also the first animal virus to be discovered followed by the discovery of the first human infecting virus, the Yellow fever virus.

An important breakthrough in the field of virology and molecular biology came with the discovery of bacteriophages i.e., viruses that infect bacteria. Bacteriophages were independently discovered by two notable scientists, namely Twort and d'Herelle (18,19). Bacteriophages are currently being used in the field of molecular biology as vehicles for gene deliveries, phage display, bacterial bio-sensing devices and even biofilm growth control (20). In the field of human clinical biology, phages are being used for the treatment of skin infections caused by bacteria, otitis externa, cholera, and certain lung infections (21). Currently, viruses are also being researched in terms of their development as effective therapeutics. For example, Oncolytic viruses specifically infect and damage tumor cells and are minimally toxic to other cells. Zika virus has demonstrated a unique oncolytic potential against aggressive glioblastoma stem cells and may become a possible therapy against brain tumors in the future (22). There are many clinically important viruses that were discovered between the 1920s and 1960s including the mumps virus, poliovirus, influenza virus, dengue virus and many others.

Another revolution was attained in the field of virology when a new class of viruses, the retrovirus, was discovered in the 1960s. Retroviruses possess RNA as their genetic material and employ an enzyme called reverse transcriptase to synthesize DNA copies from their RNA genome (12). Retroviruses are responsible for a huge disease load in humans. Several cancers are caused due to infection by retroviruses like the Rous sarcoma virus or human T-lymphotropic virus type 1 (HTLV-I). Another major disease linked to retroviruses is Acquired Immunodeficiency Syndrome (AIDS), caused by the human immunodeficiency virus (HIV). Retroviruses are responsible for many diseases in humans, but they have been utilized in many clinical applications. For example, retrovirus vectors have been in use for many years now to attain stable gene transfer— a molecular technique which is widely utilized in gene therapy (23).

Certain viruses identified over the course of time were the root cause of many epidemics. For example, in 1932, the influenza virus was first isolated in a laboratory (24). Several members of this influenza group of viruses have been noted to be responsible for many epidemics and pandemics throughout the course of history. It is generally agreed that the first influenza pandemic outbreak happened in 1580, however, some researchers

place the first outbreak in 1510. The 1580 pandemic originated in Asia and then reached Africa and Europe, subsequently spreading to America (25,26). Since 1700, influenza pandemics have caused a huge number of human fatalities, where three pandemics, namely the 1918 influenza flu, Hong Kong flu and Asian flu, claimed millions of lives (25). Other major diseases like smallpox, measles, poliomyelitis, and AIDS have claimed many millions of human lives in the past. Similarly, pandemics of 21st century like SARS, MERS (27) and COVID-19 were caused by different members of the coronavirus family.

Viroids

In 1971, Theodor O. Diener discovered a type of sub-viral pathogen which causes a devastating disease in potato plants known as potato spindle tuber disease. He later coined the name 'Viroids' for such an infectious agent (3). It was initially believed that the underlying causative agent of the potato spindle tuber disease was a virus, however, further experimentation with the extracts from the infected plants proved that the agent was a free RNA and not a virion, as previously expected (3, 28). The infected plant extracts when treated with ribonuclease, destroyed the infectivity of the infectious agent, whereas incubation with deoxyribonucleases or proteases did not affect its infectivity.

In 1974, it was established that the viroid RNA doesn't code for any proteins (29) however it was only in 1978 that the nucleotide sequence and secondary structures of the potato spindle tuber viroid were decoded (4). Viroids are pathogenic to plants. The viroid genome doesn't code for any protein and its pathogenicity is attributed to the host RNA silencing pathways (30). Current knowledge about viroid structure and its pathogenicity was dealt with tactfully in a recent review article (31).

Virusoids

Randles and group reported the first virusoid in 1981, associated with the Velvet tobacco mottle virus. They isolated it from the Australian tobacco plant (*Nicotiana velutina)* and called it a 'viroid like RNA' (32). Virusoids are known to infect plants. Like viroids, the pathogenicity of virusoids is attributed to host RNA silencing mechanisms (6).

CLASSIFICATION

The International Committee on Taxonomy of Viruses (ICTV) is the sole body which is now responsible for taxonomic classification of viruses, viroids and satRNAs along with other sub-viral agents like prions.

Viruses

Viruses have been conventionally classified by various criteria. For example, viruses have been classified based on their capsid structure, presence of an outer envelope, genetic material, disease caused, host species etc. One of the initial methods involved classifying them by their genetic material. This method was developed in the 1970s by David Baltimore (15,33) and is summarized in Table 1.

Viruses with double stranded (ds) DNA genomes parasitize a great majority of prokaryotes, followed by a significant number of single stranded (ss) DNA viruses. Most of the viruses which infect eukaryotes possess the RNA genome, however, relatively few viruses with RNA genomes are known to infect prokaryotes (34).

The ICTV has been classifying viruses since 1966. It uses an array of characteristics like type of nucleic acids, number of proteins coded, virion size, presence or absence of capsid and many more parameters to classify viruses. (35).

The ICTV has recently recognized that the taxonomy which it developed can be extended in order to project the evolutionary relationships between viruses which are distantly related. Hence, the ICTV has now changed its code in order to allow a 15 rank hierarchy, which is very similar to the Linnaean taxonomic system. 8 primary and 7 derivative ranks are used in this system. The eight principal ranks include four ranks which were already being used as described above (order, family, genus, and species) and four are new, i.e., realm, kingdom, phylum and class. The seven derivative ranks are derived from principal ranks, of which 'subfamily' was already in use in the previous ICTV system. Only primary rank, which doesn't have a derivative rank, is 'species' as no conclusive definition of 'subspecies' could be reached (36). The viruses are currently classified in six

Table 1. Baltimore classification of viruses. ss: single stranded; ds: double stranded; RT: Reverse transcriptase.

Group	Group Name	mRNA generation mechanism
I	dsDNA	Transcription takes place directly from ds DNA.
II	ssDNA	DNA is made ds by replication before the transcription.
III	dsRNA	mRNA is produced from genomic RNA
IV	ssRNA (+)	Replication to generate the negative strand from positive strand. Negative strand is transcribed.
V	ssRNA (-)	Negative strand is directly transcribed.
VI	RT ssRNA	RT is used to generate DNA from genomic RNA. This DNA is used for transcription.
VII	RT dsDNA	DNA is transcribed to generate mRNA. This mRNA is reverse transcribed to generate DNA.

realms and the complete taxonomic details can be accessed through the ICTV master list (https://talk.ictvonline.org/files/master-species-lists/m/msl/12314).

Viroids

The potato spindle tuber viroid (PSTVd) was the first viroid identified by Diener in the early 1970s (3). Another viroid, the Avocado sun blotch viroid (ASBVd), showed remarkable self-cleaving properties through hammerhead ribozymes, thus behaving like catalytic RNAs. Based on all the evidence procured from the studies on PSTVd and ASBVd, two viroid families, *Pospiviroidae* and *Avsunviroidae*, were proposed (28,37,38). The use of next generation sequencing technologies has enabled the researchers to identify new viroids (39,40). As per the ICTV, 33 viroid species are officially recognized (41,42).

Until today, 5 viroid species have been grouped in 3 genera under family *Avsunviroidae* (41) whereas 28 viroid species have been grouped in 5 genera under family *Pospiviroidae* (42). The members of *Avsunviroidae* exclusively infect dicots, whereas the members of *Pospiviroidae* infects dicots and some monocots (41,42).

In *Pospiviroidae*, the viroid replication is localized to the nucleus and is facilitated by a Class-III RNase mediated cleavage of the dsRNA structure, whereas in *Avsunviroidae*, replication takes place in the plastids, mainly the chloroplasts, using the self-cleaving properties of the hammerhead ribozyme RNA motif. The members of *Pospiviroidae* exhibit relatively low sequence diversity among themselves whereas in *Avsunviroidae*, high mutation rates have led to a complex array of sequence diversity (38,43).

Virusoids

As per the 9th ICTV report (44), a total of nine small circular satRNAs or virusoids are known and a possible member i.e. Cherry small circular viroid-like RNA is proposed. These nine officially recognized virusoids have been placed in three groups. The first of these groups concerns circular satRNAs associated with viruses belonging to the *Secoviridae* family having 3 virusoid members, whereas the second group involves one virusoid associated with viruses belonging to the *Luteoviridae* family. The last group involves virusoids associated with viruses belonging to the genus *Sobemovirus* and has 5 virusoids.

Most virusoids infect plants but the Hepatitis Delta Virus (HDV), which infect humans was previously labelled as a virusoid by some researchers. HDV has a circular RNA genome and uses Hepatitis B virus as a HV but as its genome size is relatively large (~1700 nucleotides), it doesn't strictly fulfil the requirements to be a virusoid (45). Moreover, the ICTV has classified HDV as a bona fide virus (44).

ORIGIN AND EVOLUTION

Viruses

Viruses can neither multiply nor carry out essential metabolic processes outside living cells, hence their origin still poses a dilemma to virologists. As viruses are entirely dependent on living cells, the origin and evolution of both viruses and cells seems to be intertwined (46).

Three hypotheses (namely the Virus-first hypothesis, Escape hypothesis and Reduction hypothesis) have been put forward to explain the origin of viruses:

Virus-first hypothesis

d'Herelle first claimed that viruses appeared before cells (47). Others claimed that most viruses (apart from dsRNA and negative-strand RNA viruses) originated within the primordial pool (15). As per this study, positive-strand RNA viruses descended directly from the primordial RNA-protein world and reverse-transcribing elements provided a means for transition to the DNA world.

This hypothesis is strengthened by two observations. First, RNA is currently considered as the first replicating molecule by many biologists rather than DNA. Second, ribozymes i.e., RNA molecules having enzymatic properties of catalyzing chemical reactions, are reported in the literature. These two observations point towards a possibility that these self-replicating RNA molecules originated first, even before the first cells, and then developed the ability to infect the cells, which originated later (48).

Further, a considerable portion of all viral genomes is made up of genetic sequences that lack cellular homologues and hence point towards an exclusive origin of viruses (49). However, as of today, all viruses need a cellular machinery to replicate which necessitates the presence of cells and hence this hypothesis is widely questioned.

Escape hypothesis

According to this hypothesis, viruses were initially a part of cell and are derived from fragments of cellular RNA or DNA or both, such as plasmids. These fragments escaped cellular control, acquired a protein coat and became independent structures which were capable of infecting other cells. These viruses further evolved by robbing the genes from other cells by the horizontal gene transfer mechanism (46,50).

Reduction hypothesis

This hypothesis labels viruses as 'reduced' parasitic organisms. Initially, two autonomous organisms might have entered into a symbiotic relationship with each other but over time the dependence of one might have grown more and more such that it became parasitic in nature. This parasitic organism then lost its genes which were once considered essential. As a result, they lost their ability to replicate and became obligatory intracellular parasites or 'viruses' (48,50).

Both the escape and the reduction hypothesis can be considered as 'cell-first' hypothesis as they advocate for the presence of a free-living cell before the origin of viruses. However, these Cell-first hypotheses cannot explain the presence of genetic sequences in viral genomes which lack the cellular homologues (49).

It is not possible to select any of these hypotheses as the exact mechanism. The 'Virus-first' hypothesis explains the lack of clear cellular homologues. However, ribozymes in HDV are related to the human ribozyme CPEB3, both structurally and biochemically. This observation points towards a possible origin of HDV from human transcriptome, hence advocating for the escape hypothesis (51).

Nucleocytoplasmic large DNA viruses (NCDLVs) support the reduction hypothesis. Members of this group, especially the mimivirus and poxvirus, are relatively more complex and hence they depend less on their hosts. The Mimivirus has a huge genome of 1.2 million base pairs as compared to other viruses. Similarly, the poxvirus carries many viral enzymes which allows it to produce functional mRNAs in the cytoplasm of the host cell (48,52).

In our view, strong evidence exists in favor of all three hypotheses. It is possible that different viruses must have originated distinctly using different strategies and evolved further. This conclusion is further backed by a recent study (53) which showed that major virion proteins evolved at 20 independent occasions. In some of these cases, the ancestry could be traced to the cellular proteins. Krupovic and Koonin hence inferred that some viruses could have descended from the primordial RNA world and most others evolved on multiple occasions by recruiting diverse proteins of the host cell which later became major components of the virion.

Viruses evolve just like cellular life. They undergo genetic recombination (insertion of gene fragments), genome re-assortment (replacement of genetic segments from a related virus) and point mutations where the 'fittest' mutants quickly outnumber the others (54). Viral mutation rates depend upon an array of factors involving genome type, intrinsic polymerase fidelity, presence or absence of fidelity mechanisms, editing by deaminases encoded by hosts, other host dependent factors that include an unbalanced nucleotide pool or levels of reactive oxygen species (55).

As the number of viruses is extremely large, it is beyond the scope of the current work to enumerate most of the evolutionary phenomena and other mechanisms observed among distinct classes of viruses. Therefore, influenza viruses are used as a model example to demonstrate the three mechanisms of viral evolution.

Influenza viruses are among the most notorious viruses when it comes to causing epidemics and pandemics in the history of the human race. Since 1510, 14 pandemics have been directly linked to influenza viruses, with the 1918 pandemic being the deadliest which claimed about 50 million lives worldwide (26,56). However, a recent re-assessment study of 1918 pandemic placed the number of global deaths at around 15 million (57).

The Influenza A virus (IAV) contains three proteins in its membranes, namely, hemagglutinin (HA), neuraminidase (NA) and a proton channel (M2). HA helps in the binding of the virus to the sialic acid receptor on the host cell membrane and fusion of the cell membrane with the virus. The NA cleaves the sialic acid residues and other conjugates from the newly assembled virus, facilitating its spread to other cells. As HA and NA facilitate the binding and cleaving of viruses, they play an extremely crucial role in the determination of host specificity (58). There are 16 HA subtypes which are sorted in two major groups (59). Similarly, there are 9 major subtypes of NA which are sorted in 3 groups. Two new subtypes have been recently described in bats for both HA and NA, i.e., H17, H18 and N10, N11 respectively (60).

The best strategy for IAVs (or any other parasite) to thrive is to evade the immune system of the host but at the same time preserving its ability to interact and infect host cells. Like other viruses, IAVs also employ all the 3 mechanisms to evolve and evade the immune system (58). Point mutations that change the amino acids in the antigenic portions of HA and NA can provide selective advantage to the virus (e.g., by helping the virus to better evade the immune system) and this has been labelled as antigenic drift. When re-assortment occurs in genes coding for HA and/or NA between two or more viruses in a host cell, it leads to the emergence of new progeny viruses, and this is called antigenic shift (Figure 1) (56,61).

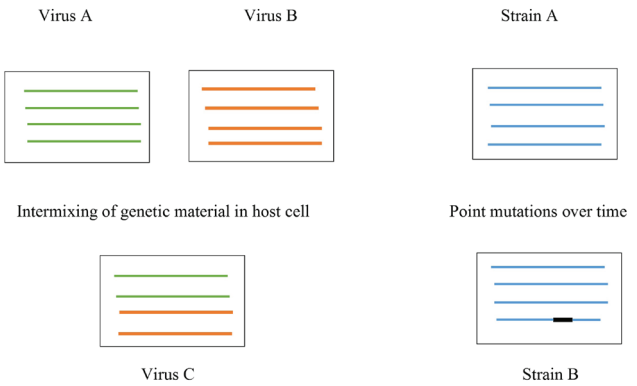

Figure 1. Antigenic shift (leading to the generation of Virus C) vs Antigenic drift (leading to the formation of a new strain of the virus).

Antigenic drift can occur in any subtype of influenza virus, however it often occurs in human IAVs due to which the IAV vaccination has a limited effect on human population, hence necessitating flu shots in every flu season. As a consequence, it kills tens of thousands of humans and adds an economic burden of USD 50 billion in the USA alone (62). If an amino acid substitution in HA or NA helps a virus escape the host immune system, it provides a fitness benefit, as a result of which, it might replace the circulating strain and emerge as a novel epidemic strain which can even lead to an epidemic or pandemic (63). It has been proposed that the surface proteins of influenza virus responsible for the 1918 pandemic drifted more rapidly which made it more lethal when compared to other viruses (64).

There are eight RNA segments in the genome of IAVs. When two related IAVs co-infect one host, there is a possibility of 256 combinations for shuffling and re-assortment. When HA or NA or both are exchanged, antigenic shift takes place leading to the emergence of a new IAV altogether, which can allow the virus to spill between the species, having the potential to cause pandemics (56). Most IAV associated pandemics are a result of re-assortment including the H2N2 virus of 1957, H3N2 of 1968 and the somewhat recent pandemic of 2009 involving the H1N1 virus (65).

The 2009 pandemic comprehensively explains the kind of evolution IAVs go through. The emergence of the H1N1 virus of 2009 involved multiple re-assortment events. A triple re-assortment event, involving three influenza viruses namely, 'classical swine' H1N1, 'human seasonal' H3N2 and North American avian influenza virus, led to the emergence of the North American 'triple reassortant' H1N2 virus in swine, which again re-assorted with the 'Eurasian-avian like' swine H1N1 influenza virus leading to the generation of the 2009 H1N1 virus which spilled over to humans causing the pandemic (66,67).

Though a rare event, recombination in IAVs occurs by two mechanisms. Non-homologous recombination (occurs between two different RNA fragments) and by the extremely rare homologous recombination which causes template switching when RNA is being replicated by the polymerase (58). The A/seal/Mass/1/80 influenza virus (H7N7) mutants contains an insertion in HA genes which is 60 nucleotides long in length and is most probably a result of a non-homologous recombination event between nucleoprotein gene and the HA gene of the same virus. This evolutionary event led to increased pathogenicity in chickens and broadened the range of host cells that the virus can infect (68). Although rare in influenza viruses, recombination is commonly encountered in natural evolution of poliovirus strains (69).

Viroids

Earlier propositions suggested that viroids might be introns that somehow escaped from the host RNAs. Such propositions were based on their shared similarities with the introns. The way viroids self-replicate resembles the process of self-splicing in introns (70). Viroids like PSTVd share nucleotide sequence similarities with the group I and group II introns (although the new bioinformatics tools put a question mark on the significance of this sequence similarity). Another theory suggested their possible origin from transposable elements, but the evidence is scarce (71,72). Moreover, many other viroids like ASBVd did not fit into this theory (38,73).

There are several features that make viroids suitable as reminiscent of the pre-cellular world. Their small size could have been a way to survive and escape their extinction due to the error-prone process of replication (74-77). Most of them possess GC rich sequences which is suggestive of relatively higher replication fidelity as GC pairs exhibit greater thermodynamic stability relative to AU pairs (78).

Their circular nature aided by the rolling circle mode of replication would have ensured complete end to end replication without loss of genetic information. It is interesting to note that there are repeating units of different lengths in the viroid genome which provides structural periodicity. The presence of ribozymes and lack of protein coding ability is suggestive of their appearance before ribosomes in an 'RNA world'. All these characteristics together with the appearance of catalytic activity (to catalyze cleavage and ligation) mediated by simple hairpin and hammerhead ribozymes would have enabled viroid replication in a pre-protein world (38). Most of the evidence points towards a monophyletic origin for the viroids and all these comparisons are deduced based on phylogenetic reconstructions. However, the possibility of viroids having a polyphyletic origin cannot be dismissed entirely, as members of the family *Avsunviroidae* show nucleotide base composition different from other viroids (38).

Virusoids

Even today our knowledge about virusoids remains limited. Hence, our knowledge about their origin and evolution still remains somewhat elusive. One reason for this information scarcity may be attributed to the low number of virusoids which have been discovered to date and their recent discovery relative to the viruses.

One study has linked virusoids to Group 1 introns. As per this study (79), Group 1 introns, which are found in the genes of nuclear and mitochondrial rRNA and chloroplastic tRNA, have a 16-nucleotide consensus sequence along with three sets of complementary sequences. These hallmarks of group 1 introns are also present in virusoids (79).

It is possible that both viroids and virusoids may have a similar history of origin despite being different in aspects of encapsidation and dependence on HVs. Both share certain features like having a small nucleotide sequence of less than 400 nucleotides and presence of hammerhead ribozymes. In our view, an elaborate mechanism for their origin cannot be predicted as of now, however the possibility of origin of these virusoids from their HVs can be out rightly rejected as most of them lack any nucleotide sequence similarities with their HV.

MODE of ENTRY and REPLICATION

Viruses

Most viruses have their genetic material encased in a protein covering which is covered by a lipid bilayer in case of enveloped viruses (80). Depending upon the virus type, they either fuse directly with the plasma membranes of the cells by receptor mediated fusion or are engulfed into an endosome.

In some cases, like HIV and poliovirus, conventional understanding advocated for a direct penetration into the host cell membrane, however, development of newer techniques, like the use of specific drugs and siRNA, which selectively prevented virus entries through specific pathways, have challenged this understanding (81). For example, in the case of the poliovirus,

a drug (ionophore monensin) was used to dissipate the cellular protein gradients which suggested a pH dependent route of virus entry and challenged the traditional understanding (81,82).

In the case of enveloped viruses, the host cell surface proteins act as receptors for viruses. For example, viruses like SARS-CoV and SARS-CoV-2, have been shown to exploit the Angiotensin-converting enzyme 2 (ACE2) receptor (83). ACE2 is widely expressed in the human respiratory tract epithelium and alveolar monocytes along with other locations like the venous endothelium, small intestine cells and in renal tubule epithelial cells (83-85).

These viruses then tend to fuse their membrane with that of the cell. The fusion process is energy driven (as lipid membranes do not fuse spontaneously) and is mediated by viral envelope glycoproteins. Three classes of these proteins have been defined according to the mechanical and structural dynamics of the viral fusion proteins, but the fundamental process of viral membrane fusion remains the same for all the classes. This process involves the simultaneous engagement of viral and target membrane which is followed by hairpin formation, ultimately leading to fusion (86). The viral genome enters the cytosol through a fusion pore which then initiates the infection (86,87).

In case of non-enveloped viruses like the simian virus 40 (SV40), the exact biophysical and molecular mechanism for cytosol entry is still poorly understood, however it is clear that the endocytic pathway plays a major role. As a result of the use of endocytic pathway, membranes of many cell organelles like Golgi and endoplasmic reticulum are also penetrated by certain non-enveloped viruses (81). Interestingly, many enveloped viruses like influenza viruses, the vesicular stomatitis virus and some others use the endocytic pathway for internalization (88).

Once inside the cell, the virus hijacks the cellular machinery. Viruses completely rely on a host's protein synthesis machinery and recruit cellular ribosomes to translate viral mRNAs (89). Depending upon the genomic constitution of the viruses, newly translated structural and non-structural or catalytic proteins are then used in the assembly of virion and to replicate the genetic material of the virus (like RNA-dependent RNA polymerases or RdRps of RNA viruses) respectively. Viruses, depending upon their genomic constitution, invoke different replication mechanisms, like rolling circle replication, rolling hairpin replication, and dsDNA bidirectional replication, among others. New viral particles are produced with the viral genetic material which are now ready for a new round of infection (87,90).

In case of viruses like bacteriophages, two types of replication cycles are reported—the lytic cycle and the lysogenic cycle. The lytic cycle involves immediate transcription, replication, and release of mature phage particles soon after infection of the bacterial cells, whereas the lysogenic cycle involves the integration of the phage genome into the bacterial genome where the phage survives as a prophage till it is induced by a stimulus to replicate and get released (91).

Few viruses have evolved this ability to integrate their genomes into the host genomes, which can have multiple consequences for the host cell involving oncogenesis, gene disruption, premature cell death and even species evolution through genome inclusions of a heritable nature. In DNA viruses like the Adenovirus, SV40 and others, viral genome integration is rarely reported, whereas it is necessary for retroviruses. Incidental integration by has been reported in the measles virus, Ebola virus, rabies virus and others (92).

Viroids

After mechanical damage to the plant cell wall, viroids enter the host through the leaf epidermis and are transported to neighboring cells (43). In some cases of an infected plant, they can enter the pollen or ovule from where they get transmitted to the seed (93). If and when the seed germinates, it gives rise to an infected plant. In the case of plants infested by the members of the *Pospiviroidae*, viroid RNA is imported into the nucleus, and is copied by plant DNA-dependent RNA polymerase II, whereas in cases of plants infected by *Avsunviroidae members*, viroid RNA is imported into the chloroplast, and RNA replication is carried out by chloroplast DNA-dependent RNA polymerase (94).

In order to systemically infect the host, viroids show three types of movements. Intracellular movement is essential for import in nucleus or chloroplast for replication. Within the cell, viroid movement is independent of the cytoskeleton and seems to be receptor-mediated, specific to certain conserved sequences and/or structural motifs. After replication, the viroid exits to the cytoplasm and utilizes cell-to-cell movement to reach neighboring cells. In order to reach the neighboring cells, viroids have been shown to exploit specialized connections between plant cells called plasmodesmata which allow them to avoid crossing the plasma membrane. Long distance movement is then utilized to reach the vasculature (e.g., phloem) and invade the most distal parts of the plants (43,95).

Viroids (*Avsunviroidae*) bear self-cleaving ribozyme structures in their RNA genomes. Viroids are not encapsidated and replicate via a 'rolling-circle' mechanism in plant hosts (96). Replication involves RNA-RNA transcription which is aided by host coded DNA-dependent RNA polymerase and are often considered as parasites of the subcellular transcriptional machinery in the nucleus and chloroplasts in plants (43).

The entire process of viroid replication occurs through two different pathways: (1) In the asymmetric pathway (family *Pospiviroidae*), the monomeric circular (+) strand is repeatedly transcribed into oligomeric (-) strands. These (-) strands are transcribed to the oligomeric (+) strand by RNA polymerase. This is followed by site-specific cleavage of the (+) strand by the RNae III-like enzyme, to produce a linear monomer that is circularized by DNA ligase (97).

(2) In the symmetric pathway (family *Avsunviroidae)*, the oligomeric (-) strand, generated in the first rolling circle generated from the monomeric circular (+) strand, is cleaved and ligated in the monomeric circular (-) strand which serves as a template

for the second rolling circle producing the oligomeric (+) strand which is processed to generate monomeric (+) strands (38,97).

Virusoids

Like other satRNAs, virusoids depend upon a HV to move, transmit and infect cells (6). Once inside the cell, a complex interaction occurs between the virusoid, HV and the host cell for the virusoid's replication. These interactions occur as a result of the dependence of the virusoid on the HV for replication and the dependence of the HV on the host cell for replication (98). Virusoids replicate in the cytoplasm of the host cell and use transcription and processing machinery, which is in part encoded by the host cells and in part by the HV (94). Structural and sequence motifs in RNA are used by virusoids to signal the HV and host cell to replicate and encapsidate it (99). Owing to the circular genome, the rolling circle mechanism is used for RNA replication. A hammerhead self-cleavage reaction is involved in the production of positive and negative monomeric RNA strands as a part of the rolling circle mechanism (100). Once replicated, the HV encapsidates the virusoid 'genome'.

The three entities show very different modes of infection. On one hand, where viruses utilize either endocytosis or receptor mediated fusion, viroids enter a host either through a damaged cell wall, infected pollen or through an infected ovule which becomes seed upon fertilization. However, the transmission from pollen to seeds is not observed in all the viroids (101). Utilizing the three different movements, viroids then infect the entire host. Virusoids movements are dependent upon HVs, and they invade the cell which is being infected by the HV. Once in the cytoplasm, virusoids show the ribozyme activity for replication. This is in contrast to viroids, which replicate either in nucleus or chloroplast.

DISCUSSION

Viruses and other sub-viral particles seem to have been with their cellular counterparts since the very beginning. Owing to the discovery of viruses before other sub-viral particles and a relatively higher impact on humans, a lot more is known about them as compared to others. In-spite of this, we still do not have a clear mechanism for the origin of viruses. It is possible that different viruses originated separately by utilizing different strategies (as explained by the Virus-first hypothesis, Escape hypothesis and Reduction hypothesis) and evolved further. It can also be speculated that all viruses originated through a mechanism which still needs to be discovered. The presence of different kinds of genomes and the ability to infect almost all cellular forms (15) point towards the intricate evolution that viruses must have undergone to achieve these extensive capabilities

The current case of the COVID-19 pandemic shows how mutations in the genome of related viruses can have huge repercussions for humanity. In spite of using the same ACE2 receptor (83), three different coronaviruses, i.e., HCoV-NL63, SARS-CoV and SARS-CoV-2 have very different infectivity and mortality rates. This shows that sharing common receptors by related viruses need not have similar outcomes. This capability would have been achieved through evolution at different levels which involves complex dynamics between primary and intermediary hosts as well as the environmental conditions alongside opportunistic infection probabilities. Epidemics and pandemics from recent history and the present show that in spite of knowing much about viruses, we are not fully capable of controlling pandemics, and hence a greater amount of support is needed for research as well as for the healthcare sector.

Owing to a much simpler structure of viroids than viruses, it is possible that they originated before viruses. There are other clues like small size, high GC content in genome and others which show that viroids must be an important component of a pre-cellular world (74).

Virusoids totally depend upon HVs to infect cells and move among them, and two scenarios are possible. Either virusoids were always dependent on HVs and originated only after the origin of viruses or it is possible that these are two independently evolved entities. It is possible that through opportunistic co-infection of similar cells, virusoids started depending upon HVs, lost their protein coding abilities and became totally dependent on viruses. The latter observation is supported by the fact that viruses and virusoids don't have nucleotide sequence homology. Furthermore, discovery of a virusoid associated with the rice yellow mottle virus, which codes for a 16 kDa protein, also supports the latter scenario (9).

Both virusoids and viroids infect plants, have similar genome size, both use the rolling circle mechanism for replication and exhibit hammerhead cleavage reaction (14,96,100). These properties suggest a probable relation between them which calls for further study.

It is of utmost importance to understand the pathogenicity of viroids and virusoids better. Since there is limited to no natural resistance in plants against the viroid infections, it has become a necessity to try and develop resistant varieties of susceptible plants. Over the years, though scientists have achieved considerable success in generating plants that show delayed occurrence of symptoms and the severity of the symptoms has also been reduced significantly (102), there is a long way to go.

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