Symbiosis, 26 (1999) 327–345
Balaban, Philadelphia/Rehovot

Single-Stranded RNA Viruses of Plants Reminiscent of Early RNA Endosymbionts

E.M.J. JASPARS
Institute of Molecular Plant Sciences, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands, Tel.: +31-71-5274778, Fax: +31-71-5274537, E-mail. jaspars@chem.leidenuniv.nl

Received October 25, 1998; Accepted March 24, 1999

Abstract

A hypothesis is advanced which defines RNA viruses of eukaryotes primarily as endosymbiotic organelles originating from prebiotic self-replicating RNA molecules. It is supposed that spore production was developed in cases where vertical transmission was hampered. In its simplest form the spore is a virion consisting of a messenger-sense RNA molecule covered with many copies of a coat protein. The coat protein is not essential for horizontal spreading. Horizontal transmission would transform RNA endosymbionts into disease agents known as viruses. A number of findings support this hypothesis, viz.: 1. Presence of vertically transmitted double-stranded RNAs in many healthy fungi, algae and higher plants. 2. Occurrence of these double-stranded RNAs in structures which either resemble the replication complexes of positive-stranded RNA viruses or the protein shells of double-stranded RNA viruses. 3. Beneficial effects for organisms harboring such RNAs. 4. Ability of separate parts of multipartite RNA genomes of virion-producing plant viruses to replicate in single cells as double-stranded RNAs in replication complexes. 5. The intermediate position of alfamo- and ilarviruses. Their naked virion RNAs are unfit for horizontal transmission. The synthesis of messenger-sense RNA molecules of these viruses is strongly dependent on the presence of coat protein as an inducer. Later in evolution this synthesis would have become constitutive, the only remaining function of the coat protein being the formation of a protective capsid.

Keywords: RNA endosymbionts, origin RNA viruses, double-stranded RNA, RNA agents, replication complexes, multipartite RNA genomes, replicating RNA, alfamoviruses, alfalfa mosaic virus, ilarviruses

0334-5114/99/$05.50

1. Introduction

Viruses are generally omitted from classifications of living organisms (Woese et al., 1990; Margulis, 1993 and 1996) because they are not autopoietic, which means that they cannot replicate independently. They need a host cell which they penetrate and from which they steal building stones and use biosynthetic machinery in order to perform their own replication. Viruses are almost exclusively seen from the view-point of obligate molecular parasitism. Their origin could lie either in the degeneration of intracellular parasitic organisms or in accidentally acquired replicational autonomy of RNA or DNA cellular elements (Matthews, 1991; Strauss et al., 1996).

With the discovery of catalytic activity of RNA molecules, the possibility of a prebiotic RNA world has become a subject of attractive speculation over the last fifteen years (Gilbert, 1986; see articles in Gesteland and Atkins, 1993). It created the option of autopoiesis for RNA molecules and the idea that present-day RNA viruses and viroids are descendants from early autopoietic ones (Watson et al., 1987; Diener, 1989; Matthews, 1991; Atkins, 1993; Strauss et al., 1996). In accordance with Occam's razor, RNA replication would not have been invented a second time in the course of evolution; the simpler hypothesis being that present RNA replicons inside higher organisms descend from prebiotic "free-living" molecules. But even from this modern view on viral origin, RNA viruses are at best considered as "molecular fossils", always with the nasty tinge of parasitism.

Whereas some RNA molecules are thought to have evolved into DNA chromosomes, and proteins and membranes would have been invented as sophisticated tools for replication and protective compartmentalization, respectively, other RNA molecules would simply profit from these inventions and continue their existence as parasites. There is one exception to this "negative" view on viruses. This is the genomic tag hypothesis (Weiner and Maizels, 1987; Maizels and Weiner, 1993) which says that viral RNAs, which have sometimes tRNA-like 3' ends, are comparable to the predecessors of present chromosomes.

In this report I will go one step further and conceive the RNA viruses of plants as organelles and place them within the serial endosymbiosis theory as coined by Taylor (1974 and 1976) and extended and strongly promoted by Margulis (1993).

2. Single-stranded RNA viruses of plants

Prebiotic RNA catalysis was already envisaged by Woese (1967), Orgel (1968) and Crick (1968) in the sixties, but until Cech and Altman with their

coworkers had demonstrated the specific catalytic activity of RNA molecules in the early eighties (for reviews see Gesteland and Atkins, 1993), nobody had thought functional RNA catalysis to be still with us. Generally spoken, proteins have taken over the role of enzymes. However, more and more examples of functional RNA catalysis have been described in recent years (Noller et al., 1992; Symons, 1992). Similarly, present RNA viruses could still have traits that inform us about their origin and evolution, "more than commonly is appreciated" (Maizels and Weiner, 1993).

The number of plant virus species that has been characterized must now be close to one thousand (Murphy et al., 1995). It is remarkable that, probably, three quarters of them have single-stranded RNA of messenger (defined as plus or positive) polarity in the virions (Zaitlin and Hull, 1987). Both picorna-like and alpha-like supergroups of RNA viruses are represented in plants, and among the representatives of these supergroups many bipartite and tripartite RNA genomes occur, respectively (Goldbach et al., 1991; Goldbach and De Haan, 1994). Quite unique for plant viruses is the phenomenon that the parts of the bi- and tripartite genomes are in separate capsids. This would be highly inefficient, if insects or other vectors were not in many cases transmitting large quantities of virions.

Even more remarkable is that the larger part (RNA 1) of the bipartite *Comoviridae* genomes, and the combination of the larger two parts (RNAs 1 and 2) of the tripartite *Bromoviridae* genomes, are able to replicate independently in isolated protoplasts. This is due to the fact that the viral subunits for the RNA-dependent RNA polymerase are encoded by the larger RNAs. The capsid proteins as well as the proteins needed for cell-to-cell transport of the virus in the plant are encoded by the smaller RNAs, i.e. RNA 2 and RNA 3, respectively. So, the infections of protoplasts by the larger RNAs only, are incomplete infections which do not produce virions (Goldbach et al., 1980; Robinson et al., 1980; Kiberstis et al., 1981; Nassuth et al., 1981; Nitta et al., 1988). Several other groups of plant viruses with multipartite genomes show this behavior of replication of the incomplete genome in protoplasts, or even in whole plants (e.g. members of the genera *Tobravirus* and *Hordeivirus*; see Murphy et al., 1995).

3. What is an RNA virus? The *Vicia* and *Cryphonectria* agents

Rather than the virion, the intracellular replication complex is the true virus (Cornuet, 1987), the agent that replicates. We have defined earlier the replication complex of a positive-stranded RNA virus as an assembly consisting of double-stranded RNA, viral RNA polymerase and enclosing membrane structure (De Graaff and Jaspars, 1994). The virion, which is often called

"virus", is in fact a positive-stranded copy of the genome, condensed with a protective viral protein to a compact structure, a spore, meant to spread the infection to other cells of the same individual and to other individuals. Incomplete infections, as mentioned already, do not produce virions, but the cells contain replication complexes that synthesize viral RNA. If these were present in a zygote, and if their replication was in balance with mitotic division, they would be a kind of organelles or endosymbionts. In virological terms, they would spread vertically. No virions for horizontal spread would be necessary to survive.

Is this a real possibility? At my knowledge, nobody has ever tried to infect a zygote with an incomplete RNA viral genome, or has grown a plant from an incompletely infected protoplast. However, it has been shown that such organelle-like agents exist in the kingdoms of plants and fungi. Investigations have focussed on two replicating RNA agents, viz. the agent associated with maternally inherited male sterility in broad bean (*Vicia faba*) (Lefebvre et al., 1990) and the agent causing hypovirulence of the chestnut blight fungus *Cryphonectria parasitica* (Fahima et al., 1993; Hillman et al., 1994). Both agents consist of a cytoplasmic membrane-associated complex of double-stranded RNA and an RNA-dependent RNA polymerase. No related virions are found in cells harboring these agents; they are vertically transmitted and do not cause proper diseases. Rather, the effects they have on their hosts are to be considered as beneficial. The *Vicia* agent prevents self-fertilization (Dulieu et al., 1998), whereas the *Cryphonectria* agent abolishes the killing action of its host (Nuss, 1992). On the one hand, these agents are useful organelles, on the other hand, they are similar to replication complexes of RNA viruses, or, better, to the RNA viruses themselves, in a non-virion producing state. This raises interesting questions about the origin and the so-called parasitic nature of RNA viruses.

4. The RNA world inside eukaryotic cells

Are the *Vigna* and *Cryphonectria* agents exceptions? I think not. Not many workers have been looking for double-stranded RNA in apparently healthy organisms. In case this has been done, well-defined double-stranded RNA species have been found quite often, both in multicellular green algae (Ishihara et al., 1992) and in higher plants (Nameth and Dodds, 1985; Wakarchuk and Hamilton, 1985; Fairbanks et al., 1988; Valverde et al., 1990; Zabalgogeazcoa and Gildow, 1992; Dodds, 1993; Fukuhara et al., 1993; Schoen et al., 1998), notably in edible plants, which means in cultivars that have been subjected to a long process of selection for size and productivity. In yeast a cytoplasmic non-encapsidated double-stranded RNA "W" has been found, which under nitrogen

starvation produces many copies of a single-stranded 20S RNA species that is not encapsidated either (Widner et al., 1991; Rodriguez-Cousiño and Esteban, 1992). Recently, two replicating double-stranded RNA species in the parasitic protozoan *Cryptosporidium parvum* have been detected and partially characterized (Khramtsov et al., 1997; Khramtsov and Upton, 1998). They share an 81% identical sequence of 27 nucleotides in the 3'-noncoding region of the plus strand. Full-size single strands of plus polarity are also found. Neither the double nor the single strands seems to be encapsidated, at least not in the oocyst phase of the protozoan.

Probably, upon further investigation, many of these double-stranded RNA molecules will appear to also belong to agents such as the ones of *Vicia* and *Cryphonectria*. It is possible that many replicating RNA agents are beneficial rather than harmful to their hosts and therefore escaped attention. Possibly, a whole world of RNA organelles is present inside eukaryotic cells.

5. Similarity of RNA replication complexes and DNA-containing organelles

The RNA replication complexes, which are in fact the "living" forms of RNA viruses, share many features with the DNA containing organelles. Both are cellular components enclosed by membranes which separate them from the cytoplasm. Inside the membranes there is a genome consisting of two complementary nucleic acid strands which can replicate using their own RNA polymerase. Both RNA and DNA endosymbionts import most of their proteins from the cytoplasm, including the subunits of their RNA polymerase. Just like the nucleus, an RNA replication complex does not have its own protein synthesizing machinery and exports its messengers for translation into the cytoplasm. Mitochondria and chloroplasts synthesize only a fraction of their proteins themselves. They do not export messengers, but import the products of nuclear messengers. The same does a replication complex when it incorporates host factors into its RNA polymerase.

The replication of both RNA replication complexes and DNA containing organelles is subjected to a tight regulation so that it keeps pace with mitosis. The point where RNA and DNA compartments diverge is function. Whereas e.g. mitochondria and chloroplasts perform essential tasks in the energy supply of the cell, the role that RNA endosymbionts play in cellular metabolism is in general unclear, and may be far from uniform.

6. RNA viruses and the serial endosymbiosis theory

According to modern theories the eukaryotic cell is an assembly of

prokaryotic endosymbionts (Margulis, 1993 and 1996; Gupta and Golding, 1996), which not only form a harmonious unit but also create the possibility for its evolution into higher multicellular forms of ever increasing complexity. I propose that present-day RNA viruses are endosymbionts, the prebiotic RNA ancestors of which found a niche once living cells arose, 4 billion years ago, and evolved with these cells as useful organelles. The functions of these RNA endosymbionts may have always remained less crucial and more diverse than those of the DNA endosymbionts, so that upon changes in the environment the function of a given RNA endosymbiont could have become marginal. Such an endosymbiont could then have been lost by accidental exclusion from the zygote. Possibly, many RNA endosymbionts may have been in such a marginal position during long periods. Selective pressure has then favored those RNA endosymbionts that found a way for horizontal transmission.

7. The origin of RNA virions

Those RNA agents that encoded proteins with affinity for RNA molecules could produce nucleoproteins which could act as primitive virions, i.e. structures in which a messenger RNA molecule was more or less protected against degradation when the organism died and so had a chance to invade a living organism and start replication again. This chance would be very small and, thus, this mechanism for horizontal spreading would only work if the production of the RNA binding protein and of the single-stranded RNA were both strongly stimulated.

Such RNA molecules, which developed coat proteins and a mechanism to stimulate the synthesis of such coat proteins and of positive-stranded RNAs, could be of great profit for other RNA endosymbionts in the same cell or organism. The latter could also use these coat proteins, but could only make virions in great numbers if they could adapt to the mechanism of producing large amounts of positive-stranded RNA. In this way accidental coendosymbionts could later become dependent on each other. The coendosymbiont that became to provide the functions for horizontal spreading could lose its replicating functions in exchange for replicating signals and could become small.

This kind of coendosymbiosis explains the situation visible e.g. in the plant virus families *Comoviridae* and *Bromoviridae*, where one of the original partners is still able to replicate independently as replication complexes in single cells.

In the context of my hypothesis the situation with the *Como-* and *Bromoviridae* reminds of the two ways of life of an RNA virus, viz. the original peaceful symbiotic way of life propagated by vertical spreading, and

an aggressive way of life derived from this, in which virions are spread horizontally. The latter way could have been a phylogenetic answer to increasing difficulties in vertical spreading. In this respect it is worth mentioning that only one fifth of the known (virion producing) plant viruses are seed transmitted, and seed transmission does not necessarily imply that the zygote was infected (Johansen et al., 1994). This supports the idea that these horizontally spreading viruses are derived from the RNA endosymbionts that had problems in being vertically transmitted.

Finally, the genomes of the coendosymbionts could merge. In fact, many RNA viruses have all their genetic information in a single RNA molecule, which increases the efficiency of horizontal transmission. This is e.g. the case with the *Picornaviridae*, which are related to the *Comoviridae*, and with the genus *Tobamovirus*, which is related to the *Bromoviridae* (Goldbach, 1987). Possibly, it is the advantage of genetic flexibility through reassortment of genome parts which favors conservation of multipartite RNA genomes. However, the evolutionary significance of separate encapsidation of genome parts is not clear. What advantage would a virus have from the possibility to start incomplete infections, if they cannot be propagated? Separate encapsidation may be understood as a relict. It still shows us the direction of evolution from RNA coendosymbionts to RNA endosymbionts.

Horizontal spreading has changed the image of viruses. When their virions reach a compatible host by accidental air or water transport or with the aid of a vector they often cause shock effects. Higher plants and animals have developed host defence mechanisms of different kinds, and horizontally transmitted viruses have often invented ways to circumvent these. Multiplying in a new cytoplasmic environment means that adaptations are needed before a new harmonious symbiosis is established. But adaptation could fail and the potential endosymbiont would now behave more like an intruder or a parasite. This means that newly invaded host cells become diseased and even die. Therefore, viruses that produce virions attracted so much attention and became the subjects of the science of virology.

Many of the RNA endosymbionts which did not develop virions might still exist unnoticed. A systematic search for and characterization of vertically transmitted RNA species in plants and, possibly, animals, could yield interesting information about the origin and evolution of RNA viruses.

8. The regulatory function of the coat protein of some *Bromoviridae*

In the forgoing I have proposed that some RNA endosymbionts evolved into virion-producing, horizontally transmitted and disease-causing viruses, whereas others remained useful organelles in eukaryote cells. Within this

hypothesis the genera *Alfamovirus* and *Ilarvirus* of the family *Bromoviridae* (Rybicki, 1995) occupy an intermediate position because of the regulatory function of their coat proteins. They are in a sense more primitive as compared to the majority of virion-producing RNA viruses, which constitutively produce large amounts of virion RNAs and have virions with tight capsids.

In the classical concept of a virus particle the genetic material of the virus is protected by a protein shell (the capsid) from the destructive factors in the environment, such as nucleases. Indeed, what would a capsid or coat protein do else if the shell of a virion upon infection is left behind on the surface of the cell while the RNA molecule is penetrating, as is the case with RNA bacteriophages (Paranchych, 1975)? However, with eukaryote viruses the whole virion enters the cell. For positive-stranded RNA viruses of plants there is experimental evidence that virions are uncoated by ribosomes translating their RNA (cotranslational disassembly; for a review see Verduin, 1992). So, inside the cell the coat protein is exerting a protective function even during primary translation of the parental RNA. It could equally be that coat protein plays an essential regulatory role in a further early step of the life cycle of the virus concerning transcription or replication of the parental RNA. This is the case with the coat protein of alfamo- and ilarviruses.

Alfalfa mosaic virus, which occurs in many different strains in nature, is the best studied member of this group. The genome of about 8 kb of this virus is tripartite and is encapsidated in three bacilliform virions of different lengths determined by the lengths of the three RNA molecules (for a review see Jaspars, 1985). The capsid structure is extremely loose and exhibits holes as have been found in no other virus (Kumar et al., 1997) and through which small ribonuclease molecules can enter and destroy the RNA (Bol and Veldstra, 1969). At slightly alkaline pH the virion structure unfolds into an ill-defined nucleoprotein string (Verhagen and Bol, 1972). All this suggests that protein-protein interactions are much less strong than RNA-protein interactions. It has even been found that, if virions and free RNA molecules are mixed in solution in vitro, the RNA molecules withdraw protein subunits from the coat of the virions, so that the virion structure after some time is completely degraded (Van Vloten-Doting and Jaspars, 1972). Responsible for this unique phenomenon are binding sites at the 3' termini of the RNA molecules with a high affinity for the coat protein (Houser-Scott et al., 1994; Reusken et al., 1994). These properties of the alfalfa mosaic virions give the impression that the structural function of the coat protein is almost negligible.

On the other hand a strong regulatory function of this coat protein is evident. The mixture of the three naked genome RNAs, although it has all genetic information in a translatable form, is infectious at a level that is several orders of magnitude lower than usual for most other viral plus-strand RNAs. However, if some coat protein is added prior to inoculation, so that the high-

affinity sites of all three genome RNAs are occupied with one or a few coat protein dimers, infectivity is strongly upregulated. This phenomenon, which is called genome activation, is most probably caused by the fact that, with this virus, messenger RNA synthesis has to be induced by coat protein (Houwing and Jaspars, 1993; Houwing et al., 1998; Jaspars, 1999). The coat protein is a regulatory protein, rather than a true structural protein. The same holds for the many members of the related genus *Ilarvirus*, which also show genome activation (Francki, 1985), although, probably, their capsids are somewhat stronger (Van Vloten-Doting, 1975).

Incomplete infections of alfamo- and ilarviruses, i.e. infections caused by inocula lacking RNA 3, which encodes the coat protein, also resemble the *Vigna* and *Cryphonectria* agents in that they replicate as double-stranded RNAs and hardly produce any single-stranded transcript. The transcript production in the absence of coat protein is so low that transfer by means of positive-stranded RNA to a new host (this type of transfer has not been tried, at my knowledge, for the *Vigna* and *Cryphonectria* agents) does not lead to a new infection cycle, unless some coat protein is added to the inoculum. Probably, the naked transfected RNAs are translated, the viral RNA polymerase is formed and synthesizes minus strands, but then the infection process ceases because the lack of messenger RNAs for new polymerase molecules prevents further multiplication.

As argued above, horizontal spread by means of virions, requires large scale production of both a coat protein and single-stranded RNA of messenger polarity. In alfalfa mosaic and related viruses these two phenomena are tightly coupled. The coat protein has even more the traits of an inducer than of a protecting molecule. RNAs 1 and 2, although they have kept their primordial character to some extent, have, on the other hand, become strongly dependent on their coendosymbiont, RNA 3, for induction of messenger production.

Probably, in the related genera *Bromovirus* and *Cucumovirus* of the *Bromoviridae* the production of all materials for virions has become a constitutive property. Thus, in the artificial situation of the laboratory, when single cells are inoculated with only one of the coendosymbionts, viz. the one with the replicase genes (represented by RNAs 1 and 2), the RNA synthesizing machinery produces significant amounts of single-stranded virion RNAs (French et al., 1986; French and Ahlquist, 1987; Pacha et al., 1990; Suzuki et al., 1991; Boccard and Baulcombe, 1993), although the protein needed for encapsidation is absent. However, there is controversy at this point. In one study on brome mosaic virus the incomplete infection was found to yield about equal amounts of plus- and minus-strands, which means that the situation resembles that of alfalfa mosaic virus (March et al., 1991).

A further phenomenon reminding of the former RNA endosymbiont state of

alfalfa mosaic virus is the observation that under certain well-defined conditions (at 23°C but not at 29°C; in cowpea, but not in tobacco protoplasts) replication complexes replicate abundantly, whereas production of virions is almost absent. Apparently, even in the presence of RNA 3 which provides the potential signal for horizontal spreading by means of virions, the signal is not understood under all conditions. With tobacco streak ilarvirus we found that in completely infected cowpea protoplasts the balance replication complexes/virions is far less dependent on temperature than in alfalfa mosaic virus (unpublished data partially shown in De Graaff and Jaspars, 1994).

9. Cryptoviruses of higher plants and mycoviruses of fungi

In higher plants and fungi not only many species of non-encapsidated double-stranded RNAs, but also many species of virus-like particles have been described which contain double-stranded RNAs and are not associated with any disease (for reviews see Milne and Natsuaki, 1995; Wickner, 1996). Attempts to transmit the latter to other hosts in order to test their infectivity failed. Apparently, they are vertically transmitted. They resemble the horizontally transmitted and double-stranded RNA containing virions of *Reoviridae*, but are less complicated in that they contain a smaller number of RNA and protein species. It is worth noting that among *Reoviridae* there are quite some viruses which, depending on the host, hardly cause any disease. The "o" of reo comes from orphan; in other words: this is a virus in search for a disease.

Are the cryptoviruses and the mycoviruses "mammals that returned to the sea"? Which means: do they descent from virion-less RNA endosymbionts that developed virions for horizontal spreading and then discovered that these virions in certain hosts were an excellent means for vertical transmission, so that they abandoned the more risky horizontal task? I think not. The obvious reason is that with these agents, as with *Reoviridae* and the double-stranded RNA bacteriophage Φ6, there is no clear distinction between replication complex and virion. All RNA synthesis takes place inside nucleocapsid-like structures (Gottlieb et al., 1990; Ghabrial, 1994; Van Dijk et al., 1995; Wickner, 1996; Nibert et al., 1996; Estes, 1996). A more logical concept is to consider the virus-like particles of these agents and the RNA replication complexes as equivalent structures. Both contain double-stranded RNA and an RNA-dependent RNA polymerase. Both are subcellular structures that release messenger RNAs into the cytoplasm. The difference is that the virion-like particles of crypto- and mycoviruses do not use host-derived membranes as a cover, but a regular spherical structure made up from their own proteins. The *Reoviridae* learned how to use this spherical structure as a vehicle for

horizontal transmission. In fact they learned how to use the replication complex itself as a virion.

If virion-like particles of higher plants and fungi are RNA endosymbionts, what is their function for the cell in which they reside and multiply? Nothing is known about this, except for the encapsidated double-stranded RNAs in certain strains of *Saccharomyces cerevisiae* and *Ustilago maydis* that encode a so-called killer toxin. The toxin producing killer RNAs are satellite double-stranded RNAs that depend for their replication and encapsidation upon independently replicating mycoviruses. Strains of a fungus that produce this toxin kill cells of other strains, and thus the killer agent is of much use for the cell in which it multiplies (for a review see Ghabrial, 1994). Such interaction phenomena could also play a role in higher plants in that an RNA endosymbiont could help a plant in dominating over or, in a reverse sense, cooperating with members of other species. Attention of virologists is mostly focussed on pathological effects of plant viruses in the laboratory or in monocultures in the field. Nothing is known about the role of viruses in the well-balanced communities of wild plant species. There is still a future for plant/virus sociobiology!

10. RNA endosymbionts in prokaryotes and unicellular eukaryotes

RNA endosymbionts have contributed to the assembly of eukaryotic cells, either as free living organisms with an RNA genome, for which there are no examples left, if any ever existed, or as RNA endosymbionts inside prokaryotes. They will not have entered the eukaryote scene as free replicating molecules as they were in the prebiotic era. However, extant RNA viruses of prokaryotes have always a lytic cycle (Mindich and Lehman, 1979; Watson et al., 1987; Mindich, 1988); there is nowhere a sign of endosymbiosis. Possibly, the prokaryotes with RNA endosymbionts in them were preferentially incorporated in eukaryotic cells, so that such prokaryotes hardly exist anymore. In this respect it is noteworthy that replication complexes of plant RNA viruses are sometimes associated with chloroplasts (De Graaff and Jaspars, 1994).

Encapsidated double-stranded RNAs (virus-like particles) have been found in the parasitic protozoa *Entamoeba histolytica, Trichomonas vaginalis, Giardia lamblia,* and in *Eimeria* and *Leishmannia* species (for a review see Wang and Wang, 1991). The agent of *Giardia* behaves as an infectious virion; the others are only vertically transmitted.

No RNA viruses of unicellular green algae have been described (Van Etten et al., 1991). Ishihara et al. (1992) found species of double-stranded RNA or virus-like particles containing double-stranded RNA in many multicellular green,

brown and red algae, and these RNAs or particles were always associated with mitochondria and chloroplasts. No double-stranded RNAs were present in established culture lines of the unicellular algae *Chlorella, Euchlena* and *Chlamidomonas*. I myself was not able to find any double-stranded RNA in a laboratory strain of *Emiliania huxlei* (unpublished). Ishihara et al. suppose that under laboratory conditions unicellular algae could eliminate their double-stranded RNAs. It is indeed tempting to speculate that upon disappearance of the selective pressure of the competition with other organisms the RNA endosymbionts lose their function. Another attractive explanation for the apparent scarcity of RNA endosymbionts in unicellular eukaryotes is that during the period of evolutionary experimentation which lead from the unicellular to the multicellular state and to differentiation, and which lasted more than one billion years, those unicellular organisms that contained RNA endosymbionts were for that very reason selected to make this important step.

11. Apparent absence of RNA endosymbionts in higher animals

Noteworthy is the apparent absence of any record of non-virion producing double-stranded RNA agents in higher animals (Kumar and Carmichael, 1998). This could be an argument against the proposed universal significance of RNA endosymbionts.

I would like to see this in the light of host defence mechanisms that arose as a response to circulating virions of many kinds. In higher plants a spreading infection caused by one strain of virions often leads to immunity for a related strain (cross-protection; see Loebenstein, 1972). In other cases the plant displays a very strong reaction at the site of the first invasion by virions (hypersensitivity). Cells at the initial site become necrotic, and so the further spreading of the infection is made very difficult. At the same time other parts of the plant become resistant against infections of many kinds, viral as well as fungal or bacterial (systemic acquired resistance; see for review Sticher et al., 1997). A clear insight into the molecular mechanism of all these plant defence systems is still lacking. We have much more information about the immune systems of higher animals, particularly mammals. In addition to circulating immunoglobulins which are directed against virion proteins with great species specificity, there is a general cytoplasmic defence mechanism provoked by double-stranded RNA. Mammalian cellular physiology is profoundly affected by double-stranded RNA in the cytoplasm. Double-stranded RNA stimulates the cell indirectly (via interferon) or directly to produce the Ser/Thr protein kinase PKR and a 2',5'-oligoadenylatesynthetase. Both enzymes are activated by binding double-stranded RNA. The active phosphorylated form of PKR

phosphorylates in its turn other proteins such as the initiation factor eIF-2α which has a strong negative effect on the initiation of protein synthesis. The activated oligoadenylate synthetase polymerizes ATP to oligo(A) with the aberrant 2',5' phosphodiester bond. The 2',5'-oligo(A) activates ribonuclease L which degrades cellular and viral RNAs (for a review see Kumar and Carmichael, 1998). Both double-stranded RNA-induced pathways have strong antiviral effects. Evidence is accumulating that the PKR pathway is also acting in higher plants (Langland et al., 1995, 1996 and 1998).

Viruses have found ways to counteract or circumvent the double-stranded RNA-induced host defence mechanisms (see the above mentioned review), but the mammalian cytoplasm could have become a too harsh environment for RNA endosymbionts to thrive. Whereas horizontal transmission by virion production was a way for RNA endosymbionts to escape from dying out as a result of exclusion from the zygote, reverse transcription and integration in the nuclear genome could have become a way to continue vertical transmission in the cells of higher animals as endogenous DNA sequences. Viral sequences, especially retroviral sequences, have indeed been found in the nuclear genomes of higher animals. *Retroviridae*, of which human immunodeficiency virus is the best studied species nowadays (for reviews see Coffin, 1996; Luciw, 1996) are RNA viruses with virions containing a diploid single-stranded genome of messenger polarity and a reverse transcriptase. They pass part of their life cycle as DNA proviruses integrated in the genome of their hosts. New single-stranded RNA copies are made by transcription from the proviral genome and are then either translated or encapsidated in the cytoplasm. Remarkably, many retroviral sequences have been found as stable genes in the nuclear genomes of all classes of vertebrates (Herniou et al., 1998). These endogenous viruses never yield infectious virions. In general, they have no pathogenic effects on their hosts. To the contrary, in some cases beneficial effects on host defence against exogenous viruses has been demonstrated (Coffin, 1996).

Estimates exists that as much as 0.1% of the human genome consists of retroviral sequences (for a review see Patience et al., 1997). The transfer of the genetic information of an RNA endosymbiont to the nuclear genome may not at all be an exceptional phenomenon, if one considers the assumption that many genes of DNA endosymbionts have done the same. The genome of mammalian mitochondria has become exceptionally small. Margulis (1993) supposes that the DNA-less undulipodia (which are eukaryotic flagella and cilia) and the mitotic apparatus are derived from a spirochete endosymbiont of which part of the DNA is now in the nucleus.

Despite the hostile environment of the cytoplasm of the mammalian cell, a plethora of virion-producing RNA viruses is able to multiply in this environment. Therefore I do expect that truly endosymbiotic RNA agents will be found in higher animals as they were in plants. Plant virologists often subject

whole plants to molecular studies. This is different from animal molecular virology where mostly cultured cell lines are used. From these the RNA endosymbionts could have disappeared, as they could have done from unicellular algae cultured in the laboratory (see above).

Acknowledgements

I thank Ms. C.J. Houwing and Drs. P. van de Putte and E.W. de Vrind-de Jong for discussion and Dr. M.J. van der Kooij for growing and harvesting a large batch of *Emiliania huxlei*. The work in my laboratory on viral RNA polymerase and RNA genome activation is supported in part by the "Stichting Bevordering Fundamenteel Onderzoek Plantevirussen".

REFERENCES

Atkins, J.F. 1993. Contemporary RNA genomes. In: *The RNA World*. R.F. Gesteland and J.F. Atkins, eds. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 535–556.

Boccard, F. and Baulcombe, D. 1993. Mutational analysis of *cis*-acting sequences and gene function in RNA 3 of cucumber mosaic virus. *Virology* **193**: 563–578.

Bol, J.F. and Veldstra, H. 1969. Degradation of alfalfa mosaic virus by pancreatic ribonuclease. *Virology* **37**: 74–85.

Coffin, J.M. 1996. *Retroviridae*: the viruses and their replication. In: *Fundamental Virology*, Ed. 3. B.N. Fields, D.M. Knipe, P.M. Howley, R.M. Chanock, J.L. Melnick, T.P. Monath, B. Roizman, and S.E. Straus, eds. Lippincott-Raven, Philadelphia, PA, pp. 763–843.

Cornuet, P. 1987. *Eléments de Virologie Végétale*. INRA, Paris, p. 83.

Crick, F.H.C. 1968. The origin of the genetic code. *Journal of Molecular Biology* **38**: 367–379.

De Graaff, M. and Jaspars, E.M.J. 1994. Plant viral RNA synthesis in cell-free systems. *Annual Review of Phytopathology* **32**: 311–335.

Diener, T.O. 1989. Circular RNAs: relics of precellular evolution? *Proceedings of the National Academy of Sciences of the USA* **86**: 9370–9374.

Dodds, J.A. 1993. dsRNA in diagnosis. In: *Diagnosis of Plant Virus Diseases*. R.E.F. Matthews, ed. CRC Press, Boca Raton, FL, pp. 273–294.

Dulieu, P., Penin, F., Dulieu, H., and Gautheron, D.C. 1998. Purification of virus-like particles from male-sterile *Vicia faba* and detection by elisa in crude leaf extracts. *Plant Science* **56**: 9–14.

Estes, M.K. 1996. Rotaviruses and their replication. In: *Fundamental Virology*, Ed. 3. B.N. Fields, D.M. Knipe, P.M. Howley, R.M. Chanock, J.L. Melnick, T.P. Monath, B. Roizman, and S.E. Straus, eds. Lippincott-Raven, Philadelphia, PA, pp. 731–761.

Fahima, T., Kazmierczak, P., Hansen, D.R., Pfeiffer, P., and Van Alfen, N.K. 1993. Membrane-associated replication of an unencapsidated double-strand RNA of the fungus, *Cryphonectria parasitica*. *Virology* **195**: 81–89.

Fairbanks, D.J., Smith, S.E., and Brown, J.K. 1988. Inheritance of large mitochondrial RNA's in alfalfa. *Theoretical and Applied Genetics* **76**: 619–622.

Francki, R.I.B. 1985. The viruses and their taxonomy. In: *The Plant Viruses*. Vol. 1. *Polyhedral Virions with Tripartite Genomes*. R.I.B. Francki, ed. Plenum Press, New York, NY, pp. 1–18.

French, R., Janda, M., and Ahlquist, P. 1986. Bacterial gene inserted in an engineered RNA virus: efficient expression in monocotyledonous plant cells. *Science* **231**: 1294–1297.

French, R. and Ahlquist, P. 1987. Intercistronic as well as terminal sequences are required for efficient amplification of brome mosaic virus RNA3. *Journal of Virology* **61**: 1457–1465.

Fukuhara, T., Moriyama, H., Pak, J.Y., Hyakutake, H., and Nitta, T. 1993. Enigmatic double-stranded RNA in Japonica rice. *Plant Molecular Biology* **21**: 1121–1130.

Gesteland, R.F. and Atkins, J.F., eds. 1993. *The RNA World*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Ghabrial, S.A. 1994. New developments in fungal virology. *Advances in Virus Research* **43**: 303–388.

Gilbert, W. 1986. The RNA world. *Nature* **319**: 618.

Goldbach, R. 1987. Genome similarities between plant and animal RNA viruses. *Microbiological Sciences* **4**: 197–202.

Goldbach, R., Rezelman, G., and Van Kammen, A. 1980. Independent replication and expression of B-component RNA of cowpea mosaic virus. *Nature* **286**: 297–299.

Goldbach, R., Le Gall, O., and Wellink, J. 1991. Alpha-like viruses in plants. *Seminars in Virology* **2**: 19–25.

Goldbach, R. and De Haan, P. 1994. RNA viral supergroups and the evolution of RNA viruses. In: *The Evolutionary Biology of Viruses*. S.S. Morse, ed. Raven Press, New York, NY, pp. 105–119.

Gottlieb, P., Strassman, J., Qiao, X., Frucht, A., and Mindich, L. 1990. In vitro replication, packaging, and transcription of the segmented double-stranded RNA genome of bacteriophage ϕ6: studies with procapsids assembled from plasmid-encoded proteins. *Journal of Bacteriology* **172**: 5774–5782.

Gupta, R.S. and Golding, G.B. 1996. The origin of the eucaryotic cell. *Trends in Biochemical Sciences* **21**: 166–170.

Herniou, E., Martin, J., Miller, K., Cook, J., Wilkinson, M., and Tristem, M. 1998. Retroviral diversity and distribution in vertebrates. *Journal of Virology* **72**: 5955–5966.

Hillman, B.I., Halpern, B.T., and Brown, M.P. 1994. A viral dsRNA element of the chestnut blight fungus with a distinct genetic organization. *Virology* **201**: 241–250.

Houser-Scott, F., Baer, M.L., Liem, K.F., Cai, J.-M., and Gehrke, L. 1994. Nucleotide sequence and structural determinants of specific binding of coat protein or coat protein peptides to the 3' untranslated region of alfalfa mosaic virus RNA 4. *Journal of Virology* **68**: 2194–2205.

Houwing, C.J. and Jaspars, E.M.J. 1993. Coat protein stimulates replication complexes of alfalfa mosaic virus to produce virion RNAs *in vitro*. *Biochimie* **75**: 617–621.

Houwing, C.J., Van de Putte, P., and Jaspars, E.M.J. 1998. Regulation of single-strand RNA synthesis of alfalfa mosaic virus in non-transgenic cowpea protoplasts by the viral coat protein. *Archives of Virology* **143**: 489–500.

Ishihara, J., Pak, J.Y., Fukuhara, T., and Nitta, T. 1992. Association of particles that contain double-stranded RNAs with algal chloroplasts and mitochondria. *Planta* **187**: 475–482.

Jaspars, E.M.J. 1985. Interaction of alfalfa mosaic virus nucleic acid and protein. In: *Molecular Plant Virology*. Vol. I. J.W. Davies, ed. CRC Press, Boca Raton, FL, pp. 155–221.

Jaspars, E.M.J. 1999. Genome activation in alfamo- and ilarviruses. *Archives of Virology* (in press).

Johansen, E., Edwards, M.C., and Hampton, R.O. 1994. Seed transmission of viruses: current perspectives. *Annual Review of Phytopathology* **32**: 363–386.

Khramtsov, N.V., Woods, K.M., Nesterenko, M.V., Dykstra, C.C., and Upton, S.J. 1997. Virus-like, double-stranded RNAs in the parasitic protozoan *Cryptosporidium parvum*. *Molecular Microbiology* **26**: 289–300.

Khramtsov, N.V. and Upton, S.J. 1998. High-temperature inducible cell-free transcription and replication of double-stranded RNAs within the parasitic protozoan *Cryptosporidium parvum*. *Virology* **245**: 331–337.

Kiberstis, P.A., Loesch-Fries, L.S., and Hall, T.C. 1981. Viral protein synthesis in barley protoplasts inoculated with native and fractionated brome mosaic virus RNA. *Virology* **112**: 804–808.

Kumar, A., Reddy, V.S., Yusibov, V., Chipman, P.R., Hata, Y., Fita, I., Fukuyama, K., Rossmann, M.G., Loesch-Fries, L.S., Baker, T.S., and Johnson, J.E. 1997. The structure of alfalfa mosaic virus capsid protein assembled as a T=1 icosahedral particle at 4.0-Å resolution. *Journal of Virology* **71**: 7911–7916.

Kumar, M. and Carmichael, G.G. 1998. Antisense RNA: function and fate of duplex RNA in cells of higher eukaryotes. *Microbiology and Molecular Biology Reviews* **62**: 1415–1434.

Langland, J.O., Jin, S., Jacobs, B.L., and Roth, D.A. 1995. Identification of a plant-encoded analog of PKR, the mammalian double-stranded RNA-dependent protein kinase. *Plant Physiology* **108**: 1259–1267.

Langland, J.O., Langland, L.A., Browning, K.S., and Roth, D.A. 1996. Phosphorylation of plant eukaryotic initiation factor-2 by the plant-encoded double-stranded RNA-dependent protein kinase, pPKR, and inhibition of protein synthesis *in vitro*. *The Journal of Biological Chemistry* **271**: 4539–4544.

Langland, J., Langland, L., and Roth, D. 1998. Differential localization and accumulation of the plant double stranded RNA-dependent protein kinase during virus infection. *Plant Physiology and Biochemistry* **36**: 395–400.

Lefebvre, A., Scalla, R., and Pfeiffer, P. 1990. The double-stranded RNA associated with the '447' cytoplasmic male sterility in *Vicia faba* is packaged together with its replicase in cytoplasmic membranous vesicles. *Plant Molecular Biology* **14**: 477–490.

Loebenstein, G. 1972. Inhibition, interference and acquired resistance during infection. In: *Principles and Techniques in Plant Virology*. C.I. Kado and H.O. Agrawal, eds. Van Nostrand Reinhold, New York, NY, pp. 32–61.

Luciw, P.A. 1996. Human immunodeficiency viruses and their replication. In: *Fundamental Virology*, Ed. 3. B.N. Fields, D.M. Knipe, P.M. Howley, R.M. Chanock, J.L. Melnick, T.P. Monath, B. Roizman, and S.E. Straus, eds. Lippincott-Raven, Philadelphia, PA, pp. 845–916.

Maizels, N. and Weiner, A.M. 1993. The genomic tag hypothesis: modern viruses as

molecular fossils of ancient strategies for genomic replication. In: *The RNA World*. R.F. Gesteland and J.F. Atkins, eds. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 577–602.

March, L.E., Huntley, C.C., Pogue, G.P., Connell, J.P., and Hall, T.C. 1991. Regulation of (+):(–)-strand asymmetry in replication of brome mosaic virus RNA. *Virology* **182**: 76–83.

Margulis, L. 1993. *Symbiosis in Cell Evolution. Microbial Communities in the Archean and Proterozoic Eons*, Ed. 2. Freeman, New York, NY.

Margulis, L. 1996. Archaeal-eubacterial mergers in the origin of eukarya: phylogenetic classification of life. *Proceedings of the National Academy of Sciences of the USA* **93**: 1071–1076.

Matthews, R.E.F. 1991. *Plant Virology*, Ed. 3. Academic Press, San Diego, CA.

Milne, R.G. and Natsuaki, T. 1995. Cryptoviruses. In: *Pathogenesis and Host Specificity in Plant Diseases. Histopathological, Biochemical, Genetic and Molecular Bases*. Vol. III: *Viruses and Viroids*. R.P. Singh, U.S. Singh, and K. Kohmoto, eds. Elsevier, Oxford, pp. 239–247.

Mindich, L. 1988. Bacteriophage ϕ6: a unique virus having a lipid-containing membrane and a genome composed of three dsRNA segments. *Advances in Virus Research* **35**: 137–176.

Mindich, L. and Lehman, J. 1979. Cell wall lysin as a component of the bacteriophage ϕ6 virion. *Journal of Virology* **30**: 489–496.

Murphy, F.A., Fauquet, C.M., Bishop, D.H.L., Ghabrial, S.A., Jarvis, A.W., Martelli, G.P., Mayo, M.A., and Summers, M.D., eds. 1995. *Virus Taxonomy. Classification and Nomenclature of Viruses. Sixth Report of the International Committee on Taxonomy of Viruses*. Springer, Wien. (*Archives of Virology* [Suppl.] 10).

Nameth, S.T. and Dodds, J.A. 1985. Double-stranded RNAs detected in cucurbit varieties not inoculated with viruses. *Phytopathology* **75**: 1293 (Abstract).

Nassuth, A., Alblas, F., and Bol, J.F. 1981. Localization of genetic information involved in the replication of alfalfa mosaic virus. *The Journal of General Virology* **53**: 207–214.

Nibert, M.L., Schiff, L.A., and Fields, B.N. 1996. Reoviruses and their replication. In: *Fundamental Virology*. Ed. 3. B.N. Fields, D.M. Knipe, P.M. Howley, R.M. Chanock, J.L. Melnick, T.P. Monath, B. Roizman, and S.E. Straus, eds. Lippincott-Raven, Philadelphia, PA, pp. 691–730.

Nitta, N., Takanami, Y., Kuwata, S., and Kubo, S. 1988. Inoculation with RNAs 1 and 2 of cucumber mosaic virus induces viral RNA replicase activity in tobacco mesophyll protoplasts. *The Journal of General Virology* **69**: 2695–2700.

Noller, H.F., Hoffarth, V., and Zimniak, L. 1992. Unusual resistance of peptidyl transferase to protein extraction procedures. *Science* **256**: 1416–1419.

Nuss, D.L. 1992. Biological control of chestnut blight: an example of virus-mediated attenuation of fungal pathogenesis. *Microbiological Reviews* **56**: 561–576.

Orgel, L.E. 1968. Evolution of the genetic apparatus. *Journal of Molecular Biology* **38**: 381–393.

Pacha, R.F., Allison, R.F., and Ahlquist, P. 1990. *cis*-Acting sequences required for *in vivo* amplification of genomic RNA3 are organized differently in related bromoviruses. *Virology* **174**: 436–443.

Paranchych, W. 1975. Attachment, ejection and penetration stages of the RNA phage infectious process. In: *RNA Phages*. N.D. Zinder, ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 85–111.

Patience, C., Wilkinson, D.A., and Weiss, R.A. 1997. Our retroviral heritage. *Trends in Genetics* **13**: 116–120.

Reusken, C.B.E.M., Neeleman, L., and Bol, J.F. 1994. The 3'-untranslated region of alfalfa mosaic virus RNA 3 contains at least two independent binding sites for viral coat protein. *Nucleic Acids Research* **22**: 1346–1353.

Robinson, D.J., Barker, H., Harrison, B.D., and Mayo, M.A. 1980. Replication of RNA-1 of tomato black ring virus independently of RNA-2. *The Journal of General Virology* **51**: 317–326.

Rodriguez-Cousiño, N. and Esteban, R. 1992. Both yeast W double-stranded RNA and its single-stranded form 20S RNA are linear. *Nucleic Acids Research* **20**: 2761–2766.

Rybicki, E.P. 1995. Bromoviridae. In: *Virus Taxonomy. Classification and Nomenclature of Viruses. Sixth Report of the International Committee on Taxonomy of Viruses*. F.A. Murphy, C.M. Fauquet, D.H.L. Bishop, S.A. Ghabrial, A.W. Jarvis, G.P. Martelli, M.A. Mayo, and M.D. Summers, eds. Springer, Wien, pp. 450–457.

Schoen, C.D., Miglino, R., Jelkmann, W., and Leone, G. 1998. Molecular cloning of dsRNAs associated with strawberry mottle virus. *Acta Horticulturae* **471**: 51–55.

Sticher, L., Mauch-Mani, B., and Métraux, J.P. 1997. Systemic acquired resistance. *Annual Review of Phytopathology* **35**: 235–270.

Strauss, E.G., Strauss, J.H., and Levine, A.J. 1996. Virus evolution. In: *Fundamental Virology*, Ed. 3. B.N. Fields, D.M. Knipe, P.M. Howley, R.M. Chanock, J.L. Melnick, T.P. Monath, B. Roizman, and S.E. Straus, eds. Lippincott-Raven, Philadelphia, PA, pp. 141–159.

Suzuki, M., Kuwata, S., Kataoka, J., Masuta, C., Nitta, N., and Takanami, Y. 1991. Functional analysis of deletion mutants of cucumber mosaic virus RNA3 using an *in vitro* transcription system. *Virology* **183**: 106–113.

Symons, R.H. 1992. Small catalytic RNAs. *Annual Review of Biochemistry* **61**: 641–671.

Taylor, F.J.R. 1974. Implications and extensions of the serial endosymbiosis theory of the origin of eukaryotes. *Taxon* **23**: 229–258.

Taylor, F.J.R. 1976. Autogenous theories for the origin of eukaryotes. *Taxon* **25**: 377–390.

Valverde, R.A., Nameth, S., Abdallha, O., Al-Musa, O., Desjardins, P., and Dodds, A. 1990. Indigenous double-stranded RNA from pepper (*Capsicum annuum*). *Plant Science* **67**: 195–201.

Van Dijk, A.A., Frilander, M., and Bamford, D.H. 1995. Differentiation between minus- and plus-strand synthesis: polymerase activity of dsRNA bacteriophage Φ6 in an *in vitro* packaging and replication system. *Virology* **211**: 320–323.

Van Etten, J.L., Lane, L.C., and Meints, R.H. 1991. Viruses and virus-like particles of eukaryotic algae. *Microbiological Reviews* **55**: 586–620.

Van Vloten-Doting, L. 1975. Coat protein is required for infectivity of tobacco streak virus: biological equivalence of the coat proteins of tobacco streak and alfalfa mosaic viruses. *Virology* **65**: 215–225.

Van Vloten-Doting, L. and Jaspars, E.M.J. 1972. The uncoating of alfalfa mosaic virus by its own RNA. *Virology* **48**: 699–708.

Verduin, B.J.M. 1992. Early interactions between viruses and plants. *Seminars in Virology* **3**: 423–431.

Verhagen, W. and Bol, J.F. 1972. Evidence for a pH-induced structural change of alfalfa mosaic virus. *Virology* **50**: 431–439.

Wakarchuk, D.A. and Hamilton, R.I. 1985. Cellular double-stranded RNA in *Phaseolus vulgaris*. *Plant Molecular Biology* **5**: 55–63.

Wang, A.L. and Wang, C.C. 1991. Viruses of the protozoa. *Annual Review of Microbiology* **45**: 251–263.

Watson, J.D., Hopkins, N.H., Roberts, J.W., Steitz, J.A., and Weiner, A.M., eds. 1987. *Molecular Biology of the Gene*, Ed. 4. Benjamin-Cummings, Menlo Park, CA, p. 1139.

Weiner, A.M. and Maizels, N. 1987. tRNA-like structures tag the 3' ends of genomic RNA molecules for replication: implications for the origin of protein synthesis. *Proceedings of the National Academy of Sciences of the USA* **84**: 7383–7387.

Wickner, R.B. 1996. Viruses of yeasts, fungi and parasitic microorganisms. In: *Fundamental Virology*, Ed. 3. B.N. Fields, D.M. Knipe, P.M. Howley, R.M. Chanock, J.L. Melnick, T.P. Monath, B. Roizman, and S.E. Straus, eds. Lippincott-Raven, Philadelphia, PA, pp. 425–453.

Widner, W.R., Matsumoto, Y., and Wickner, R.B. 1991. Is 20S RNA naked? *Molecular and Cellular Biology* **11**: 2905–2908.

Woese, C.R. 1967. *The Genetic Code*. Harper & Row, New York, p. 186.

Woese, C.R., Kandler, O., and Wheelis, M.L. 1990. Towards a natural system of organisms: proposal for the domains archaea, bacteria, and eucarya. *Proceedings of the National Academy of Sciences of the USA* **87**: 4576–4579.

Zabalgogeazcoa, I.A. and Gildow, F.E. 1992. Double-stranded ribonucleic acid in 'Barsoy' barley. *Plant Science* **83**: 187–194.

Zaitlin, M. and Hull, R. 1987. Plant virus-host interactions. *Annual Review of Plant Physiology* **38**: 291–315.