Viruses of marine algae

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The study of plant viruses is an advanced field, but largely confined to those which infect horticultural or crop plants. Some attention has also been given to weed or wild plants which might serve as reservoirs for these viruses. Because of this emphasis on 'economic' virology, there is little documentation of viruses which must exist that infect lower plants, particularly in this context, the algae. There is a body of information on viruses of fresh-water blue-green algae, again triggered by the economic relationship between these algae and water quality. The viruses of these photosynthesizing procaryotes are phage-like particles which contain double-stranded DNA. Their properties and biology have been reviewed (Brown[1], Padan and Shilo[2]).

Evidence for viruses of eucaryotic algae is less satisfactory but not completely absent and has also been reviewed (Andrews[3], Brown[1], Hoffman and Stanker[4], Lemke[5]). A virus with properties in common with tobacco mosaic virus, including the possession of a single-stranded RNA genome, has been isolated and characterized from *Chara corallina* and has been shown to be infectious (Gibbs et al.[6], Skotnicki et al.[7]). This represents the only proof to date of a virus infecting a eucaryotic alga, in this case from fresh-water. *Chara* spp. are extremely complex algae, and TMV is such a ubiquitous virus that this is unlikely to become a model alga-virus system. It is, nevertheless, an important example because it establishes that viruses do infect eucaryotic algae and can be expected if looked for elsewhere.

Another line of evidence for viruses in eucaryotic algae is the accumulation over recent years of observations by electron microscopy of virus-like particles (VLPs) in thin sections of numerous algae, both fresh-water and marine. The algae in question are arranged taxonomically in the table, together with the size of the reported VLPs and an indication of which are marine.

These observations have not normally been the result of a deliberate search for VLPs, but have been incidental to other studies. A few of the algae are available in culture, but most of the reports deal with field-collected materials no longer at hand. The following is a summary of the observations made in these studies.

Shape, structure and size of VLPs. Except for the rod shaped VLP in *Chara corallina*, the particles are all polygonal (5- or 6-sided) in outline and consist of a multilaminate membrane-like shell enclosing core

A taxonomic list of eucaryotic algae in which virus-like particles (VLPs) have been observed

Class	Alga	Reference	VLP dimensions
Rhodophyceae	**Porphyridium purpureum*	Chapman[8,9], Chapman and Lang[10]	40 nm
	**Sirodotia tenuissima*	Lee[11]	50–60 nm
Phaeophyceae	**Ectocarpus fasciculatus*	Baker and Evans[12] Clitheroe and Evans[13]	170 nm
	**Pylaiella littoralis*	Markey[14]	130–170 nm
	**Chorda tomentosa*	Toth and Wilce[15]	170 nm
Chlorophyceae	*Cylindrocapsa geminella*	Hoffman and Stanker[4,16]	200–230 nm
	Uronema gigas	Mattox et al.[17] Dodds et al.[18]	390 nm
	Oedogonium sp.	Picket-Heaps[19]	240 nm
	Aulacomonas sp.	Swale and Belcher[20]	200–230 nm
Charophyceae	*Chara corallina*	Gibbs et al.[6] Skotnicki et al.[7]	532 nm × 18 nm
Prasinophyceae	**Pyramimonas orientalis*	Moestrup and Thomsen[21]	60 nm and 200 nm
	**Micromonas pussila*	Mayer[22], Pienaar[23]	129 nm, 120 nm
	**Platymonas* sp.	Pearson and Norris[24]	57 nm
Haptophyceae	**Hymenomonas carterae*	Pienaar[23]	65 nm
	**Chrysochromulina mantoniae*	Manton and Leadbeater[25]	22 nm
	**Coccolithus huxleyi*	Manton and Leadbeater[25]	
Chrysophyceae	**Hydrurus foetidus*	Hoffman[26]	50–60 nm
	Endosymbiont of *Peridium balticum*	Tomas and Cox[27] Tomas et al.[28]	35–45 nm
Chryptophyceae	**Cryptomonas* sp.	Pienaar[23]	100 nm and 240 nm × 120 nm

*Marine algae.

material. One particle was further enclosed in a pear-shaped sac (Pienaar[23]). The reported diameters for the VLPs are from 22 to 390 nm. All particles are about the same size for a given alga except for 2 reports of more than 1-sized particle in the same cell (Moestrup and Thomsen[21], Pienaar[23]). Certain size clusters are apparent in the Rhodophyceae (40–60 nm), the Phaeophyceae (170 nm) and the Chlorophyceae (200–240 nm).

Location in the cell. VLPs have been reported in the nucleus, in the cytoplasm or in both locations (Tomas et al.[28]). Quasi-crystalline arrangements of VLPs have been observed.

Inclusion bodies. Cells from cultures containing VLPs sometimes show features not associated with normal cells. These include amorphous (Lee[11], Markey[14]) or concentric (Chapman and Lang[10]) inclusions in the nucleus or cytoplasm and unusual membranous activity in the cytoplasm (Markey[14]). There has been speculation that such inclusions may be involved in VLP synthesis.

Effects on cells. Cells containing VLPs have been described as moribund with the expectation that such cells will ultimately lyse. Lysis has been reported (Mayer[22]). Some specific effects are proliferation of chloroplasts (Pienaar[23]), loss of the outer nuclear membrane (Pearson and Norris[24]), general disruption of organelles (Pickett-Heaps[19]), a failure to produce a cell wall (Toth and Wilce[15]), and abnormal development of surface scales (Pienaar[23]). Several of the reports which infer that the cytoplasm is the location of VLPs observe that infected cells lacked a nucleus (Clitheroe and Evans[13], Hoffman and Stanker[4], Mattox et al.[17], Moestrup and Thomsen[21], Pienaar[23], Swale and Belcher[20]). One explanation for this, suggested by several micrographs, is that the VLPs were formed at the expense of the nucleus. In contrast to these adverse effects, other reports indicate that infected cells had a normal appearance except for the presence of VLPs (Pearson and Norris[24]).

Location in tissues. The vegetative cells of multicellular algae appear not to contain VLPs. The exception is the VLP in *Sirodotia* which was also observed in more than 1 stage in the algal life cycle. The more common observation is that VLPs are only found in reproductive cells or the germlings produced from them (Clitheroe and Evans[13], Hoffman and Stanker[4], Markey[14], Pickett-Heaps[19], Toth and Wilce[15]). Such cells may be distinguishable from normal cells in the light microscope (Pickett-Heaps[19]).

Frequency of cells containing VLPs. Cells containing VLPs are generally infrequent in infected cultures, although 30% of *Platymonas* cells were visibly infected (Pearson and Norris[24]). It is more usual to find that VLPs are present in only one or two cells out of hundreds examined (Hoffman[26], Moestrup and Thomsen[21]). Re-detection of VLPs in other cultures or collections of the same alga has been negative in some searches (Markey[14]) but positive in others (Chapman[9], Lee[11]).

Types of algae with VLPs. The algae listed cover a wide range of vegetative types from many taxonomic groups. Noticeably absent are the larger fucoid and laminarean algae.

What is still lacking in this summary of results is the evidence to establish these particles as true viruses, and an understanding of the part they play in the biology and ecology of the algae in which they are found. It is difficult to make this kind of progress because most of the specimens are not in culture, virus frequency is generally low, vegetative cells do not appear to be likely sources of VLPs for purification, and simple diagnostic tests which could be used to detect infections and to monitor virus purifications quantitatively have not been developed. This final practical problem has been a necessary prelude to progress in other fields of virology. Bacterial, animal, and plant virology have all relied heavily on plaque and lesion assays, or serological tests, for these purposes.

Some of these problems have been partly overcome in 1 system, the VLP in the filamentous fresh-water alga *Uronema gigas.* Recent studies (Cole and Dodds[29]) have expanded the results reported earlier (Dodds et al.[18]) and the purified VLP has the following virus-like properties. It has a 390-nm diameter capsid attached to 10% of which is a tail-like appendage 1.0 µm in length. The large size and appearance of the VLPs make them instantly recognizable when negatively stained and examined in the electron microscope, and this is the assay that has been used in a semi-quantitative way to monitor purifications and determinations of biochemical properties. The partially purified VLP has a sedimentation coefficient of 6300 S and a buoyant density in sucrose of 1.32 g/ml. Double-stranded DNA molecules with a mol. wt of between 8×10^6 and 72×10^6 daltons have been associated with the particle which also contains at least 10 different protein molecules ranging in mol. wt from 26,000 to 64,000 daltons. The VLPs are found only in young filaments and are gradually released into the growth medium, with no indication of a sudden lysis, which makes it necessary to grow cultures for 2 months in litre quantities to produce enough VLPs for purification. Attempts to produce healthy cultures from isolated single germlings failed, but the cultures that were selected showed a range of capacity to release VLPs. A pathological effect of virus production was observed since low-yielding cultures grew better than high-yielding cultures. Failure to obtain VLP-free cultures has prevented attempts to demonstrate infectivity.

The foregoing illustrates that one of the priorities for future research, the determination of biochemical

properties of the particles, is feasible. Modern analytical tools, notably polyacrylamide gel electrophoresis of nucleic acids and proteins and visualization of nucleic acids and VLPs in the electron microscope, require only small amounts of purified viruses and such quantities can probably be obtained. From such studies will come answers to the question of relatedness of VLPs. The similarity in sizes of VLPs from specific groups of algae noted earlier is the only indication that some of the VLPs may be more closely related to each other than to those of other groups.

Future biochemical characterizations may also help resolve an intriguing possibility that the VLPs reported in algae are also viruses of other marine organisms. The basis for this suggestion is that of all the existing groups of viruses the only logical group in which to place VLPs of the type described earlier is the icosohedral cytoplasmic deoxyriboviruses (Kelly and Robertson[30]) which are known or suspected to infect many kinds of organisms including protozoa, amphibia, reptiles, mollusks and fishes. The possibility exists that algae may serve as alternate hosts for viruses of other organisms or other organisms could act as vectors of algal viruses.

The dispersal of algal viruses and the processes by which they infect healthy algae are completely unknown and are priorities for future research. Vegetative cells with thick cell walls seem unlikely candidates for sites of infection, but naked zoospores are much more attractive for this role. The observation that VLPs are most commonly seen in moribund zoospores or germlings likely to lyse and release VLPs may reflect a mechanism to ensure that quantities of VLPs are present in an aqueous environment at a time when other zoospores are available for the VLPs to encounter and infect.

A number of observations point to latency or integration as a factor in the biology of algal viruses. These include the absence of detectable VLPs in the vegetative cells of cultures which nevertheless contain VLPs in their reproductive cells, the ability of infected cultures to maintain themselves even though VLPs seem to be lysing some cells, and the ability to select subcultures which are either very low or very high producers of VLPs. The most striking observation is that germlings of *Cylindrocapsa geminella* contained VLPs only when the culture was first heat-shocked (Hoffman and Stanker[4]). A similar heat shock treatment increased the VLP productivity from *U. gigas* 6-fold. Whether integration of viral genomes is involved in any of these phenomena remains to be tested, but the observation that double-stranded DNA is the genomic nucleic acid of one of these VLPs lends support to the possibility.

A summary of these speculations is that algal VLPs may be double-stranded DNA viruses that remain latent or integrated through much of the life cycle of their algal host and are produced at a time most likely to lead to transmission. When VLPs are produced in quantity, this occurs in certain cells only and while these may show cytopathological effects and may lyse, others do not and so the host or the culture is maintained. Future work on VLP purification may be helped by learning to control the onset of VLP productivity from non-productive infected cells. How significant this kind of an interaction may be to the biology and, particularly the pathology of algae, remains to be seen; but in the meantime, a great deal of satisfaction can be obtained by the characterization of what will clearly be novel viruses. In this context, it is worth observing that the size of the VLP in *U. gigas* makes it as large or larger than the pox-viruses, generally recognized as the largest viruses known. In addition, the tail-like appendage is unique among viruses of eucaryotes. Observations of this kind should encourage phycologists and virologists to join forces and make some progress in the new field of eucaryotic algal virology.

1 R.M. Brown, Jr. Adv. Virus Res. *17*, 243 (1972).
2 E. Padan and M. Shilo, Bact. Rev. *37*, 343 (1973).
3 J.H. Andrews, Biol. Rev. *51*, 211 (1976).
4 L.R. Hoffman and L.H. Stanker, Can. J. Bot. *54*, 2827 (1976).
5 P.A. Lemke, A. Rev. Microbiol. *30*, 105 (1976).
6 A.J. Gibbs, A.H. Skotnicki, J.E. Gardiner, E.S. Walker and M. Hollings, Virology *64*, 571 (1975).
7 A.H. Skotnicki, A.J. Gibbs and N.G. Wrigley, Virology *75*, 457 (1976).
8 R.L. Chapman, J. Phycol. *8*, (suppl.), 14 (1972).
9 R.L. Chapman, J. Phycol. *9*, (suppl.), 16 (1973).
10 R.L. Chapman and N.J. Lang, J. Phycol. *9*, 117 (1973).
11 R.E. Lee, J. Cell Sci. *8*, 623 (1971).
12 J.R.J. Baker and L.V. Evans, Protoplasma *77*, 1 (1973).
13 S.B. Clitheroe and L.V. Evans, J. Ultrastruct. Res. *49*, 211 (1974).
14 D.R. Markey, Protoplasma *80*, 223 (1974).
15 R. Toth and R.T. Wilce, J. Phycol. *8*, 126 (1972).
16 L.R. Hoffman and L.H. Stanker, J. Phycol. *11*, (suppl.), 7 (1975).
17 K.R. Mattox, F. Stewart and J. Floyd, Can. J. Microbiol. *18*, 1620 (1972).
18 J.A. Dodds, J.R. Stein and S. Haber, Proc. Can. phytopath. Soc. *42*, 26 (1975).
19 J.D. Pickett-Heaps, J. Phycol. *8*, 44 (1972).
20 E.M.F. Swale and J.H. Belcher, Arch. Mikrobiol. *92*, 91 (1973).
21 D. Moestrup and H.R. Thomsen, Protoplasma *81*, 247 (1974).
22 J. Mayer, J. Phycol. *13*, (suppl.), 44 (1977).
23 R.N. Pienaar, Phycologia *15*, 185 (1976).
24 B.R. Pearson and R.E. Norris, Phycologia *13*, 5 (1974).
25 I. Manton and B.S.C. Leadbeater, Dan. Vidensk. Sel. Biol. Skr. *20* (5), 1 (1974).
26 L.R. Hoffman, J. Phycol. *14*, 110 (1978).
27 R.N. Tomas and E.R. Cox, J. Phycol. *9*, 304 (1973).
28 R.N. Tomas, G.C. Lindsay, R.S. Nairn, and E.R. Cox, J. Phycol. *9*, (suppl.), 16 (1973).
29 A. Cole and J.A. Dodds, unpublished results.
30 D.C. Kelly and J.S. Robertson, J. gen. Virol. *21*, 417 (1973).