

REVIEW

Dinoflagellates, Diatoms, and Their Viruses

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Since the first discovery of the very high virus abundance in marine environments, a number of researchers were fascinated with the world of “marine viruses”, which had previously been mostly overlooked in studies on marine ecosystems. In the present paper, the possible role of viruses infecting marine eukaryotic microalgae is enlightened, especially summarizing the most up-to-the-minute information of marine viruses infecting bloom-forming dinoflagellates and diatoms. To author’s knowledge, ~40 viruses infecting marine eukaryotic algae have been isolated and characterized to different extents. Among them, a double-stranded DNA (dsDNA) virus “HcV” and a single-stranded RNA (ssRNA) virus “HcRNAV” are the only dinoflagellate-infecting (lytic) viruses that were made into culture; their hosts are a bivalve-killing dinoflagellate *Heterocapsa circularisquama*. In this article, ecological relationship between *H. circularisquama* and its viruses is focused. On the other hand, several diatom-infecting viruses were recently isolated and partially characterized; among them, one is infectious to a pen-shaped bloom-forming diatom species *Rhizosolenia setigera*; some viruses are infectious to genus *Chaetoceros* which is one of the most abundant and diverse diatom group. Although the ecological relationships between diatoms and their viruses have not been sufficiently elucidated, viral infection is considered to be one of the significant factors affecting dynamics of diatoms in nature. Besides, both the dinoflagellate-infecting viruses and diatom-infecting viruses are so unique from the viewpoint of virus taxonomy; they are remarkably different from any other viruses ever reported. Studies on these viruses lead to an idea that ocean may be a treasury of novel viruses equipped with fascinating functions and ecological roles.

Keywords: algal viruses, dinoflagellate, diatom, *Heterocapsa circularisquama*, *Rhizosolenia setigera*, *Chaetoceros* spp., HcV, HcRNAV, RsRNAV, CsNIV, CdebDNAV

Since the first discovery of the extremely high abundance of virus-like particles (VLPs) in natural waters, the significance of aquatic viruses has been highlighted (Bergh *et al.*, 1989). Most of them are assumed to be viruses infectious to bacteria, cyanobacteria, archaea and other larger organisms (phytoplankton, zooplankton, seaweeds, crustacean, fishes, and any other aquatic organisms). So far, several studies have highlighted their possible ecological significance (Proctor and Fuhrman, 1990; Thingstad *et al.*, 1993; Suttle, 2000, 2005; Wommack and Colwell, 2000; Brussaard, 2004), but the ecological roles of individual viruses have not been sufficiently investigated. Author’s main interest is focused on the relationship between eukaryotic microalgae and their viruses.

Viruses or VLPs have been found in more than 50 species in 12 classes of eukaryotic algae (Van Etten *et al.*, 1991; Zingone, 1995; Brussaard, 2004). Noticeable reports and events (since 1972) in the history of eukaryotic algal virus study are shown in Table 1. Until 1980’s, most intensive characterization was made on *Chlorella* viruses (ex. PBCV-1) (ex. Van Etten *et al.*, 1991) and *Micromonas pusilla* virus

(MpV) (Mayer and Taylor, 1979; Waters and Chan, 1982); both are large icosahedral viruses harboring a dsDNA genome. Especially, findings of various types of *Chlorella* viruses (Reisser *et al.*, 1988a, 1988b; Van Etten *et al.*, 1991) and determination of genome structure of PBCV-1 (ex. Rohozinski *et al.*, 1989) were prominent topics of 1980’s. In 1990’s, several novel algal viruses similar in size and shape to PBCV-1 and MpV were isolated (ex. Brussaard, 2004); and the full-genome sequencing of PBCV-1 was completed (ex. Li *et al.*, 1997). They were categorized as a new virus family *Phycodnaviridae*, in which six virus genera (*Chlorovirus*, *Prasinovirus*, *Prymnesiovirus*, *Phaeovirus*, *Coccolihovirus*, and *Raphidovirus*) are included (Van Etten *et al.*, 2002; Wilson *et al.*, 2005b; Dunigan *et al.*, 2006). Viruses in culture that are infectious to marine eukaryotic algae are listed in Table 2 (this kind of tables must be periodically renewed thanks to the perpetual establishment of new algal virus cultures). In 20th century, algal virologists wondered why only large dsDNA viruses (NCLDV: nucleocytoplasmic large DNA viruses) were found in algae; which was remarkably different from the case of plant viruses where ssRNA viruses by far dominate. Since the first finding of ssRNA virus infecting a raphidophyte *Heterosigma akashiwo* (HaRNAV) (Tai *et al.*, 2003), however, several smaller viruses infecting algae have been also iso-

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Table 1. Noticeable events and reports in the history of eukaryotic algal virus studies

Year	Noticeable events and reports
1972	First isolation of a virus infecting eukaryotic chlorophyte <i>Uronema gigas</i> (Mattox and Stewart, 1972)
1978	First discovery of virus-like particles in symbiont <i>Chlorella</i> of <i>Paramecium</i> (Kawakami and Kawakami, 1978)
1979	Isolation of dsDNA virus infecting a marine prasinophyte <i>Micromonas pusilla</i> (Mayer and Taylor, 1979)
1981	Finding and further analysis of viral infection in symbiont <i>Chlorella</i> of <i>Hydra viridis</i> (Meints <i>et al.</i> , 1981; Van Etten <i>et al.</i> , 1981)
1982	Isolation of a virus infecting symbiont <i>Chlorella</i> of <i>Paramecium</i> (Van Etten <i>et al.</i> , 1982)
1982~	Intensive characterization of <i>Chlorella</i> virus PBCV-1 (ex. Rohozinski <i>et al.</i> , 1989; Van Etten <i>et al.</i> , 1991)
1989	Discovery of a huge amount of virus particles in natural waters (Bergh <i>et al.</i> , 1989)
1991	Finding of a dsDNA virus of a phaeophyte <i>Ectocarpus silliculosus</i> , EsV (Müller <i>et al.</i> , 1991)
1993	Suggestion of possible viral impact on <i>Emiliania huxleyi</i> blooms (Bratbak <i>et al.</i> , 1993)
1995	Establishment of the family <i>Phycodnaviridae</i> in “Virus Taxonomy” of ICTV (Van Etten, 1995)
1996	Suggestion of availability of DNA polymerase gene for phylogenetic study of algal DNA viruses (Chen <i>et al.</i> , 1996)
1997	Full genome sequencing of PBCV-1 (ex. Li <i>et al.</i> , 1997)
1998	The 1st Algal Virus Workshop (Bergen, Norway)
1999	Suggestion of possible use of <i>Chlorella</i> virus enzymes (Yamada <i>et al.</i> , 1999)
2000	Establishment of four new genera in the family <i>Phycodnaviridae</i> in “Virus Taxonomy of ICTV” (Van Etten, 2000) The 2nd Algal Virus Workshop (Galway, Ireland)
2001	Isolation of a dsDNA virus infecting a dinoflagellate <i>Heterocapsa circularisquama</i> (Tarutani <i>et al.</i> , 2001) Full genome sequencing of EsV (Delaroque <i>et al.</i> , 2001)
2002	Isolation of a dsDNA virus infecting <i>E. huxleyi</i> , EhV (Castberg <i>et al.</i> , 2002; Wilson <i>et al.</i> , 2002) The 3rd Algal Virus Workshop (Hiroshima, Japan)
2003	Isolation of a ssRNA virus of <i>Heterosigma akashiwo</i> (Tai <i>et al.</i> , 2003) Examination on diversity in EhV population during <i>E. huxleyi</i> bloom (Schroeder <i>et al.</i> , 2003)
2004	Isolation of a dsRNA virus infecting <i>M. pusilla</i> (Brussard <i>et al.</i> , 2004a) Isolation of a ssRNA virus infecting a diatom <i>Rhizosolenia setigera</i> (Nagasaki <i>et al.</i> , 2004a) Isolation of a ssRNA virus infecting the dinoflagellate <i>H. circularisquama</i> , HcRNAV and examination on its diversity (Tomaru <i>et al.</i> , 2004a) Examination on diversity in HaV population during <i>H. akashiwo</i> bloom (Tomaru <i>et al.</i> , 2004b)
2004~	Scultiny of ecological relationship between a haptophyte <i>Phaeocystis globosa</i> and its viruses (ex. Baudoux and Brussaard, 2005; Brussard <i>et al.</i> , 2004b, 2007)
2005	Isolation of a ssDNA virus infecting a diatom <i>Chaetoceros salsugineum</i> (Nagasaki <i>et al.</i> , 2005c) Full genome sequencing and microarray analysis of EhV (Wilson <i>et al.</i> , 2005a) Expansion of the family <i>Phycodnaviridae</i> (Wilson <i>et al.</i> , 2005b) The 4th Algal Virus Workshop (Amsterdam, Holland)
2006	Suggestion of wide distribution of RNA viruses by metagenomics methods (Culley <i>et al.</i> , 2006) Practical Workshop on Virus Ecology Methods (Plymouth, UK)
2007	Comparison of <i>Chlorella</i> viruses differing in their origin (Fitzgerald <i>et al.</i> , 2007a, 2007b, 2007c) Examination of ecological relationship between <i>H. circularisquama</i> and its viruses <i>in situ</i> (Tomaru <i>et al.</i> , 2007)
2008	Identification of two types of latent viruses in a phaeophyte <i>Feldmannia</i> sp. (Meints <i>et al.</i> , 2008) The 5th Aquatic Virus Workshop (Bancouver, Canada)

lated, and now it is well known that algal viruses are diverse in size (25 to over 220 nm), genome type (dsDNA, ssDNA, dsRNA, ssRNA), genome structure (linear, circular, fragmented or not), and genome size (4.4 to over 500 kb). Many of them are large dsDNA viruses which are categorized in the family *Phycodnaviridae* (e.g., MpV, EhV, HaV, PpV, CeV,

PoV, AaV); three are single-stranded RNA viruses apparently composed of two groups that are respectively infectious to Stramenopiles (raphidophyte and diatom: ex. HaRNAV, RsRNAV, SssRNAV) and Alveolata (dinoflagellate: ex. HcRNAV); two are ssDNA viruses infecting a diatom (CsNIV, CdebdNAV); and one is a dsRNA virus infecting a

Table 2. Viruses infecting eukaryotic algae

Virus	Host	Size (nm)	Genome	Principal references
[Viruses infecting unicellular algae]				
AaV (BtV)	<i>Aureococcus anophagefferens</i>	140	dsDNA	Garry <i>et al.</i> (1998), Gastrich <i>et al.</i> (2004)
CbV	<i>Chrysochromlina brevifilum</i>	145-170	dsDNA	Suttle and Chan (1995)
CdebDNAV	<i>Chaetoceros debilis</i> *	30	ssDNA, fragmented?	Tomaru <i>et al.</i> (2008)
CeV	<i>Chrysochromlina ericina</i>	160	dsDNA, 510 kb	Sandaa <i>et al.</i> (2001)
CgNIV	<i>Chaetoceros cf. gracilis</i> *	30	ssDNA(?), 3-4 kb	Y. Eissler (pers. comm.)
<i>Chlorella</i> virus (ex. ATCV-1, ATCV-2) (symbiont of <i>Acanthocystis turfacea</i>)	<i>Chlorella</i> SAG 3.83	140-190	dsDNA, 288 kb	Bubeck and Pfitzner (2005), Fitzgerald <i>et al.</i> (2007c)
<i>Chlorella</i> virus (ex. PBCV-1, NY-2A, (symbiont of <i>Paramecium bursaria</i>) AR158)	<i>Chlorella</i> NC64A	150-190	dsDNA, 331-369 kb	Fitzgerald <i>et al.</i> (2007b), Van Etten and Meints (1999), Van Etten <i>et al.</i> (1991, 2002), Yamada <i>et al.</i> (1999, 2006)
<i>Chlorella</i> virus (ex. MT325, FR483) (symbiont of <i>Paramecium bursaria</i>)	<i>Chlorella</i> Pbi	140-150	dsDNA, 314-321 kb	Fitzgerald <i>et al.</i> (2007a), Reisser <i>et al.</i> (1988a, 1988b), Van Etten <i>et al.</i> (1991), Yamada <i>et al.</i> (2006)
<i>Chlorella</i> virus (ex. HVCV)	<i>Chlorella</i> -like alga (symbiont of <i>Hidra viridis</i>)	170-180	dsDNA, ~200 kb	Van Etten <i>et al.</i> (1981, 1982, 1991)
CtenRNAV	<i>Chaetoceros tenuissimus</i> *	31	ssRNA	Shirai <i>et al.</i> (article in press)
CsNIV	<i>Chaetoceros salsugineum</i> *	38	(ss+ds)DNA, 6.0 kb	Nagasaki <i>et al.</i> (2005c)
CspNIV	<i>Chaetoceros cf. gracilis</i> *	25	-	Bettarel <i>et al.</i> (2005)
CsfrRNAV	<i>Chaetoceros socialis f. radians</i> *	30	RNA	Y. Tomaru (unpublished data)
EhV	<i>Emiliania huxleyi</i>	170-200	dsDNA, 410-415 kb	Allen <i>et al.</i> (2006), Castberg <i>et al.</i> (2002), Schroeder <i>et al.</i> (2003), Wilson <i>et al.</i> (2002, 2005a)
HaNIV	<i>Heterosigma akashiwo</i>	30	-	Lawrence <i>et al.</i> (2001)
HaV	<i>Heterosigma akashiwo</i>	202	dsDNA, 294 kb	Nagasaki and Yamaguchi (1997), Nagasaki <i>et al.</i> (1999, 2005b), Tarutani <i>et al.</i> (2000), Tomaru <i>et al.</i> (2004b)
HaRNAV	<i>Heterosigma akashiwo</i>	25	ssRNA, 9.1 kb	Tai <i>et al.</i> (2003), Lang <i>et al.</i> (2004)
HcRNAV	<i>Heterocapsa circularisquama</i> **	30	ssRNA, 4.4 kb	Mizumoto <i>et al.</i> (2007), Nagasaki <i>et al.</i> (2004b, 2005a, 2006), Tomaru <i>et al.</i> (2004a)
HcV	<i>Heterocapsa circularisquama</i> **	197	dsDNA, 356 kb	Tarutani <i>et al.</i> (2001), Nagasaki <i>et al.</i> (2003, 2005b, 2006)
MpRV	<i>Micromonas pusilla</i>	50-60	dsRNA, 24.6 kb	Brussaard <i>et al.</i> (2004a), Attoui <i>et al.</i> (2006)
MpV	<i>Micromonas pusilla</i>	115	dsDNA, 200 kb	Cottrell and Suttle (1991, 1995), Mayer and Taylor (1979), Waters and Chan (1982)
MpVN1	<i>Micromonas pusilla</i>	110-130	(ds?)DNA	Zingone <i>et al.</i> (2006)
MpVN2	<i>Micromonas pusilla</i>	110-130	(ds?)DNA	Zingone <i>et al.</i> (2006)
OIs1	<i>Heterosigma akashiwo</i>	30 and 80	dsDNA, 20 and 130 kb	Lawrence <i>et al.</i> (2006), J. Lawrence (unpublished data)
PgV-102P	<i>Phaeocystis globosa</i>	98	dsDNA, 176 kb	Wilson <i>et al.</i> (2006)
PgV Group I	<i>Phaeocystis globosa</i>	150	dsDNA, 466 kb	Brussaard <i>et al.</i> (2004b, 2007), Baudoux and Brussaard (2005)
PgV Group II	<i>Phaeocystis globosa</i>	100	dsDNA, 177 kb	Brussaard <i>et al.</i> (2004b, 2007), Baudoux and Brussaard (2005)
PoV	<i>Pyramimonas orientalis</i>	180-220	dsDNA, 560 kb	Sandaa <i>et al.</i> (2001)
PpV	<i>Phaeocystis pouchetii</i>	130-160	dsDNA, 485 kb	Jacobsen <i>et al.</i> (1996), Bratbak <i>et al.</i> (1998), Yan <i>et al.</i> (2005)
RsRNAV	<i>Rhizosolenia setigera</i> *	32	ssRNA, 11.2 kb	Nagasaki <i>et al.</i> (2004a), Shirai <i>et al.</i> (2006)
[Viruses infecting multicellular algae]				
EsV	<i>Ectocarpus siliculosus</i>	130-150	dsDNA, 336 kb	Lanka <i>et al.</i> (1993), Müller <i>et al.</i> (1996, 1998), Van Etten <i>et al.</i> (2002)
EfasV	<i>Ectocarpus fasciculatus</i>	135-140	dsDNA, 340 kb	Müller <i>et al.</i> (1996, 1998)
FlexV	<i>Feldmannia simplex</i>	120-150	dsDNA, 170 kb	Friess-Klebl <i>et al.</i> (1994), Müller <i>et al.</i> (1998)
FirrV	<i>Feldmannia irregularis</i>	140-170	dsDNA, 180 kb	Kapp <i>et al.</i> (1997), Müller <i>et al.</i> (1998)
FsV	<i>Feldmannia</i> species	150	dsDNA, 158 & 178 kb	Henry and Meints (1992), Müller <i>et al.</i> (1998), Meints <i>et al.</i> (2008)
HincV	<i>Hinckia hinckiae</i>	140-170	dsDNA, 220 kb	Kapp <i>et al.</i> (1997), Müller <i>et al.</i> (1998)
MclV	<i>Myriotrichia clavaeformis</i>	170-180	dsDNA, 340 kb	Kapp <i>et al.</i> (1997), Müller <i>et al.</i> (1998)
PlitV	<i>Pilayella littoralis</i>	161	dsDNA, 280 kb	Maier <i>et al.</i> (1998), Müller <i>et al.</i> (1998)

* diatom, ** dinoflagellate

prasinophyte (MpRV) (Table 2). Thus, now we realize that marine microalgae are universally exposed to attacks by various types of virus.

Among the marine eukaryotic microalgae, dinoflagellate and diatom are the most abundant and diverse groups; they are considered to be the most widespread plants on earth. They are abundant not only in marine environments but also freshwater environments (diatoms can grow even in soil and moist surfaces of rocks and plants). Their contribution to the world net primary production is remarkable and they contain a great number of species, also including various harmful bloom-forming species and toxic bloom-formers causing fisheries damages. In this paper, biology of dinoflagellate-viruses and diatom-viruses will be concisely reviewed.

Dinoflagellate viruses

The first reports of dinoflagellate-infecting dsDNA virus (HcV: *Heterocapsa circularisquama* virus) and ssRNA virus (HcRNAV: *Heterocapsa circularisquama* RNA virus) were done in 2001 and 2004, respectively. To my understanding, they are the only viruses lytic to dinoflagellates successfully made into culture so far (Tarutani *et al.*, 2001; Tomaru *et al.*, 2004a). Prior to the isolation of these viruses, viral infection in dinoflagellates had been scarcely investigated; there were only three reports concerning the VLPs observed in dinoflagellates by TEM in the 1970's (Franca, 1976; Soyer, 1978; Sicko-Goad and Walker, 1979). However, these VLPs were not made into culture thus further study was not performed. Recent investigation on VLPs (or virus-like agent having continuous transferability) of zooxanthellae species (symbiotic dinoflagellates of Coelenterata) is of great interest (Wilson *et al.*, 2001; Davy *et al.*, 2006). Just recently, Lohr *et al.* (2007) succeeded in isolating a latent virus-like agent of *Symbiodinium* sp. inducible with UV. From the viewpoint of reef ecosystem study, the new host-virus systems are of much concern. Further investigation is essential for a better understanding of the viral impact on reef ecosystems. Below noticeable characteristics of the two cultured viruses HcV and HcRNAV are summarized.

HcV: HcV is a large (170~210 nm in diameter) icosahedral virus (Fig. 1A) (Tarutani *et al.*, 2001) harboring a dsDNA genome that is ca. 356 kb in length (Nagasaki *et al.*, 2005b). This virus is propagated within the cytoplasm of the host cell where the virion is accumulated with the viroplasmic

regions (Tarutani *et al.*, 2001; Nagasaki *et al.*, 2003, 2006). Based on these features, HcV is considered to be a member in the family *Phycodnaviridae* (Wilson *et al.*, 2005b; Phylogenetic analysis on its DNA polymerase amino acid sequence is now under way). HcV infects *H. circularisquama* at a wide range of temperatures (15~30°C). The latent period and the burst size were respectively estimated at 40~56 h and 1,800~2,440 infectious units per cell, and HcV has a relatively wide strain-host range compared to HcRNAV (Nagasaki *et al.*, 2003; Tomaru *et al.*, 2004a). As in the case of most algal viruses tested, vigorously growing host culture is more sensitive to HcV than stationary phase culture (Nagasaki *et al.*, 2003). The ecological relationship between *H. circularisquama* and HcV has not been sufficiently investigated so far; HcV-like particles' appearance in natural *H. circularisquama* bloom (coexisting with cells harboring HcRNAV-like particles) was reported by Tomaru and Nagasaki (2004). Again since 2006, several strains of HcV-like virus have been successfully isolated from the *H. circularisquama* blooms in the coastal waters of Japan by using extinction dilution method. This may indicate HcV keeps being one of the viruses that dominate in *H. circularisquama* blooms and have a considerable ecological impact on the host bloom dynamics.

HcRNAV: HcRNAV is a polyhedral virus ~30 nm in diameter (Fig. 1B) harboring a genome ca. 4.4 kb long. The virus replicates in the cytoplasm of *H. circularisquama* (Tomaru *et al.*, 2004a). The latent period and the burst size were respectively estimated at 24~48 h and 3,400~21,000 infectious units per cell. Both the host and virus populations are composed of multiple types; i.e., by a cross-reactivity test, HcRNAV clones collected from the western part of Japan were roughly divided into two types (UA and CY), which showed complementary strain-specific infectivity; hence, the host strains were also divided into two types; type C hosts were only sensitive to type UA HcRNAV, and type B hosts were only sensitive to type CY HcRNAV (Tomaru *et al.*, 2004a). To summarize, multiple types of *H. circularisquama* and HcRNAV (i.e., at least two independent host-virus systems) coexisted in a given geographical area. The genomic RNA of typical strains of type CY and UA HcRNAV (HcRNAV109 and HcRNAV34, respectively) were fully sequenced and compared (DDBJ accession numbers are AB218608 and AB218609, respectively). They were ~97.0% similar to each other and each genome has two open reading frames (ORF-1 and -2) (Nagasaki *et al.*, 2005a). The 3' ends of the viral genomes lacked of poly(A) tail but was with stem-loop structures. ORF-1 is assumed to be a replicase polyprotein gene encoding at least serine protease and RNA-dependent RNA polymerase (RdRp). The best fit BLAST comparison of the serine protease domain of HcRNAV was for the Lucerne transient streak virus (BLAST e-value based on 2008 database = 4e-4) and those of the conservative RdRp domain were relatively close to the Mushroom bacilliform virus (5e-11), Poinsettia cryptic virus (1e-10) and Poinsettia latent virus (7e-10) (Nagasaki *et al.*, 2005a). These significantly low BLAST hits apparently show that HcRNAV is evolutionarily quite distant from any other land or aquatic viruses that have been genetically studied. Considering that HcRNAV is the sole ssRNA virus infecting the phylum "Alveolata", these data seem to be rational

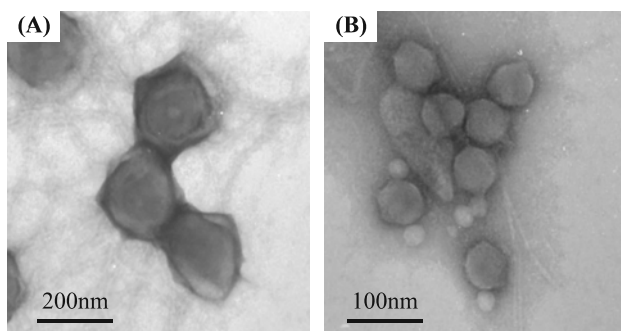


Fig. 1. Transmission electron micrographs of HcV (A) and HcRNAV (B).

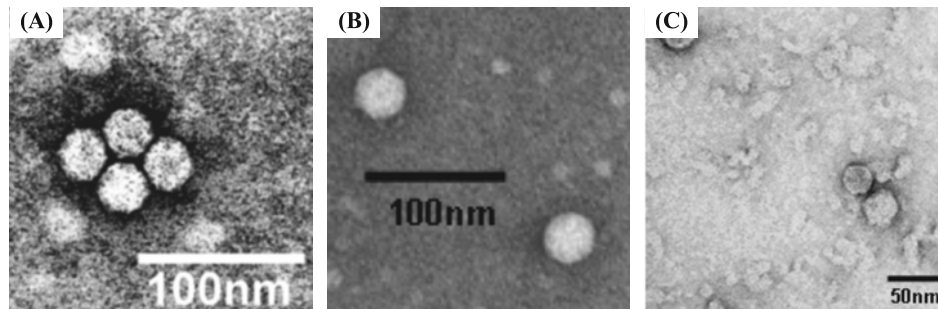


Fig. 2. Negatively stained virions of RsRNAV (A), CsNIV (B), and CdebDNAV (C).

(see below). ORF-2 codes for the viral single major structural protein (Nagasaki *et al.*, 2005a). ORF-2 has four specific regions where amino acid substitutions (between type CY and type UA) are frequently found; the tertiary structure of the capsid proteins predicted by using computer modeling supported this hypothesis; i.e., many of the amino acid substitutions were, in the model, located in regions on the outside of the viral capsid proteins exposed to the ambient water environments (Nagasaki *et al.*, 2005a). When the genome RNA of HcRNAV109 (type CY virus) was injected into 'inappropriate' host *H. circularisquama* cells (type C host) using a particle bombardment method, the intracellular virus replication occurred at a lower efficiency. In contrast, no viral replication was detected when the HcRNAV109 suspension was simply inoculated to the type C host culture (Mizumoto *et al.*, 2007). Integrating these data, the type-specific host specificity of this virus is principally determined by nano-structures on the virus surface that may affect its binding affinity to the host cell. The less efficient replication of HcRNAV in the host cell transfected with 'inappropriate' virus RNA is presumably due to the compatibility between the viral genome and the intracellular condition that is essential for virus replication.

Diatom viruses

Since the first isolation of a diatom-infecting ssRNA virus (RsRNAV: *Rhizosolenia setigera* RNA virus) by Nagasaki *et al.* (2004a), several viruses infectious to a diatom have been made into culture to date. Prior to the isolation of RsRNAV, viral infection in diatoms was almost unknown. Proctor and Fuhrman (1991) presented a TEM photograph of a diatom-like cell which harbors a number of large VLPs (~0.1 μm in diameter), which was occasionally found in a zooplankton fecal pellet. All of the cultured diatom-viruses are much smaller in size (20~40 nm) than phycodnaviruses. Considering the accumulation site of the VLPs (in the cytoplasm) and the size (Proctor and Fuhrman, 1991), however, future finding of diatom-infecting phycodnaviruses is not surprising at all. Below remarkable characteristics of the three cultured diatom-viruses are summarized.

RsRNAV: RsRNAV is a small (32 nm in diameter) polyhedral ssRNA virus (Fig. 2A) specifically infecting the bloom-forming diatom *R. setigera* (Nagasaki *et al.*, 2004a). Virion accumulation was observed within the cytoplasm but not in the nucleus. Infectivity of RsRNAV is also strain-specific, and its intra-species host specificity is diverse among virus

clones as observed in the case of other algal viruses (ex. HaV, HcRNAV) (Tarutani *et al.*, 2000; Tomaru *et al.*, 2004a, 2004b). The latent period and the burst size were estimated at <48 h and 1,100~3,100 infectious units per host cell (Nagasaki *et al.*, 2004a). RsRNAV genome is 8,877 nt, polyadenylated at 3'-end, lacking a cap structure at the 5'-end, and has two large open reading frames: they are predicted to be a replicase polyprotein gene coding for replicases (ex. RNA helicase, RdRp) and a structural polyprotein gene coding for (at least) three coat proteins (Shirai *et al.*, 2006). The deduced amino acid sequences for these ORFs respectively showed considerable similarities to the non-structural and structural proteins of a marine raphidophyte-infecting virus HaRNAV (Lang *et al.*, 2004). Phylogenetic analyses of deduced amino acid sequences of RNA helicase and RdRp domains supported the monophyly of RsRNAV, HaRNAV and a marine protist-infecting virus SssRNAV (*Schizochytrium* single-stranded RNA virus) with moderate bootstrap values of 79~83% (i.e., not at the family level). Comparison of the genome organization and sequence suggests RsRNAV is not a member of any currently defined virus family (see below).

CsNIV: The genus *Chaetoceros* is the most abundant and widespread diatom group; CsNIV (*C. salsugineum* nuclear inclusion virus) is the first cultured *Chaetoceros*-infecting virus. It is a 38 nm icosahedral virus (Fig. 2B) that replicates within the nucleus of *C. salsugineum*, which is a bloom-forming, colony-forming, and universally observed diatom species that occurs in brackish lakes and estuarine waters (Nagasaki *et al.*, 2005c). The latent period and burst size were estimated to be 12~24 h and 325 infectious units per host cell (the latter may be underestimated) (Nagasaki *et al.*, 2005c). The most noticeable feature of CsNIV is its extraordinary genome structure: the genome consists of a single molecule of covalently closed circular ssDNA (6,000 nt) as well as a segment of linear ssDNA (997 nt). The linear segment is complementary to a portion of the closed circle creating a partially double-stranded region. Sequence analysis revealed a low but significant similarity of an ORF to the replicase of bird-infecting circoviruses that have a covalently closed circular ssDNA genome. Just recently, another diatom-virus having a similar genome structure has been isolated; thus, their relationship is of great concern (Y. Tomaru, unpublished data).

CdebDNAV: CdebDNAV is another *Chaetoceros*-infecting virus, which causes lysis of the cosmopolitan species *C. debilis* Cleve (Tomaru *et al.*, 2008). It was isolated from both water

and sediment samples collected in coastal environments of western Japan. CdebDNAV is a 30 nm icosahedral virus (Fig. 2C) accumulating in the cytoplasm of *C. debilis*; its proliferation may be in the nucleus as long as TEM observation. Host specificity of CdebDNAV is also strain-specific and its infectivity is stably maintained at a wide temperature range between 20°C and -196°C (without adding any cryoprotectant). The latent period and the burst size were estimated to be 12~24 h and 55 infectious units per host cell (maybe the latter is underestimated as in the case of CsNIV). The genome is composed of several ssDNA fragments. Sequence analysis (now under way) revealed that the partial region of CdebDNAV genome showed a high similarity (E value=1e-56) to the putative replicase gene of CsNIV (see above). This result predicts the existence of ssDNA virus group infectious to genus *Chaetoceros*. The genome analysis is now under way.

CspNIV: CspNIV (*Chaetoceros* nuclear inclusion virus) is infectious to *Chaetoceros* cf. *gracilis* (Bettarel *et al.*, 2005) isolated from the Chesapeake Bay, USA, in April 2003. Transmission electron microscopy of ultrathin sections of infected *C. cf. gracilis* revealed that CspNIV proliferates within the nucleus and forms paracrystalline arrays corresponding to the alignment of icosahedral viral particles of about 25 nm diameter. The latent period of CspNIV is <24 h. The most widespread occurrence of *Chaetoceros* viruses in Chesapeake Bay was recorded in April 2003, ca. 1 mo after the winter-spring *Chaetoceros* bloom. However, results indicate that CspNIV remains infectious in surface water of the bay no longer than 1 month after the disappearance of its host. The genome structure of this virus has not been reported so far.

Implication and future view

To this end, remarkably important eukaryotic algal members, dinoflagellates and diatoms are also exposed to viral attacks. Indeed further studies are essential to elucidate the ecological implication of dinoflagellate-viruses and diatom-viruses; the following points are to be the center of attention.

Ecological roles of viruses: Changes in abundance of *H. circularisquama* and its viruses have been continuously assessed and compared from 2001 in Ago Bay, Japan (Nagasaki *et al.*, 2004b; Tomaru *et al.*, 2007). Because the occurrence of *H. circularisquama* bloom is always accompanied with specific increase in abundance of viruses lytic to *H. circularisquama*, their intimate ecological relationship is apparent. Through the survey, viral impact on *H. circularisquama* population was shown to be not only quantitative (having impact on host biomass) but also qualitative (changing the host clonal composition); i.e., HcRNAV types UA and CY showed independent dynamics both in waters and sediments (Nagasaki *et al.*, 2004b). This should have reflected the changes in host clonal composition *in situ*; i.e., abundance of each suitable host type. In addition, considering that only a small-sized bloom occurred when a considerable amount of HcRNAV remained in the sediment just prior to the blooming season of *H. circularisquama* (July-August, 2003), viral infection is considered to be one of the most significant factors determining the size and duration of the host bloom (Tomaru *et al.*, 2007). Following *H. circularisquama* blooms, the abun-

dance of HcRNAV in the water column rapidly decreased; whereas, the sediment viruses showed a much more gradual decrease; hence, sediments may be the adequate reservoir for HcRNAV (Nagasaki *et al.*, 2004b; Tomaru *et al.*, 2007).

In the case of diatom study, accumulation of ecological data is a future subject. Especially in the case of studies on viruses infecting *Chaetoceros*, accurate identification and enumeration of their hosts at the species level are almost impossible by only using optical microscopy. In future study, innovation in enumerating each species (e.g., real-time PCR) will be essential.

Intraspecies host specificity: As mentioned above, there were two HcRNAV types dominant in natural waters (Nagasaki *et al.*, 2004b; Tomaru *et al.*, 2004a). Presumably the nano-structure difference on the capsid surface may determine the intraspecies host specificity; thus, the nano-structure of virus-receptors of the two *H. circularisquama* types (B and C) are assumed to be different and determine their virus sensitivity spectra (Nagasaki *et al.*, 2005a). Still, the viral receptors of *H. circularisquama* have not been identified yet. Similar relationships between host and virus have been observed in diatom-viruses; ex. RsRNAV (Nagasaki *et al.*, 2004a), CdebDNAV (Tomaru *et al.*, 2008). Examination on the mechanism supporting the strain-specific infectivity of these viruses is of great concern.

Variety of algal viruses: BLAST results and phylogenetic trees constructed based on the amino acid sequences of the viruses introduced in the present paper made us realize the immaturity (= poverty) of marine virus sequence database; in other word, these viruses are all novel and remarkably distant from any other viruses examined so far. A few years ago, Lang *et al.* (2004) proposed a new virus family *Marnaviridae*, which includes only an ssRNA virus member infecting the bloom-forming raphidophyte *H. akashiwo* (HaRNAV). However, any of the ssRNA viruses introduced in the present paper does not belong to the family *Marnaviridae*; it is based on phylogenetic analysis results and the number of ORFs (HaRNAV has only one ORF, RsRNAV and HcRNAV has two ORFs). Further, HcRNAV is extremely distinct from HaRNAV, RsRNAV, and SssRNAV in the RdRp phylogenetic tree (Shirai *et al.*, 2006); i.e., in the RdRp tree, the positive-sense ssRNA viruses infecting Stramenopiles (RsRNAV, HaRNAV, and SssRNAV) and Alveolata (HcRNAV) were categorized into phylogenetically distant clades, which suggests a host/virus coevolution; thus, the data is assumed to be so reasonable. The phylogenetic comparison supports the hypothesis that a diverse array of ssRNA viruses exists in marine environments.

A few novel diatom-viruses are being made into culture and their feature is now under analysis. Adding data of these novel diatom viruses will further highlight the remarkable variety of algal viruses in nature.

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