$$1 = (1/z - 1)^{-1/B1}\frac{C_1}{IC_{50_1}}[1 + (PI_2C_2)^{BP2}]^{-1} + (1/z - 1)^{-1/B2}\frac{C_2}{IC_{50_2}}[1 + (PI_1C_1)^{BP1}]^{-1}$$

where $z = (a - y)/(a - d)$ is the normalized effect. In the equation for z, y is the measured p24 level in natural units, a is the level in the absence of drug, and d is the level at indefinitely high drug concentrations. The variable y defines a surface over C_1 and C_2, which represent the concentrations of the two drugs. IC_{50_1} and IC_{50_2} are the 50% inhibitory concentrations of the two drugs used separately; B_1 and B_2 are the corresponding 50%-effect slopes; BP_1 and BP_2 are slope parameters for the potentiating effects; PI_1 and PI_2 are potentiation indices for drug 1 acting on drug 2, and drug 2 acting on drug 1, respectively. For the present data, it sufficed to set $BP_1 = BP_2 = 1$ and $PI_1 = 0$. Weights for the fitting procedure were determined from the error structure of the data set itself with a Gaussian kernel windowing technique based on estimated responses.

16. J. S. Morgan, D. C. Creasey, J. A. Wright, *Biochem. Biophys. Res. Commun.* **134**, 1254 (1986).
17. S. Koenig *et al.*, *Science* **233**, 1089 (1986).
18. M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *ibid.* **224**, 497 (1984).
19. J. M. McCune *et al.*, *Cell* **53**, 55 (1988).
20. S. Gartner *et al.*, *Science* **233**, 215 (1986).
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Isolation of Virus Capable of Lysing the Brown Tide Microalga, *Aureococcus anophagefferens*

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Viruses have been hypothesized to control blooms of *Aureococcus anophagefferens* gen. et sp. nov. (Chrysophyceae), a marine phytoplankton that since 1985 has caused devastating summer blooms called "brown tide." By means of ultrafiltration methods, viruses specific to this alga were isolated from both the Great South Bay and Peconic Bay systems of Long Island, New York, during the summer bloom period of 1992. Cell lysis of healthy algal cultures was demonstrated, as well as continuing reinfection with serial transfers of cultures. Electron microscope surveys yielded images of phage-like virus particles with tails that could attach to *A. anophagefferens* cells within minutes of exposure. The isolation and cultivation of this virus highlights the need for further study of viral infection of eukaryotic algae and the potential for a better understanding of algal bloom control by viral infection.

Brown tide blooms were first documented in 1985, in Narragansett Bay, Rhode Island, in Barnegat Bay, New Jersey, and in the Peconic Bay and Great South Bay systems of Long Island, New York. These bays experienced practically simultaneous algal blooms of the eukaryotic microalga, *Aureococcus anophagefferens* gen. et sp. nov. (Chrysophyceae) (*1*). The blooms colored the water a deep, golden-brown (*2*), drastically reduced light through the water column, and caused widespread death of eelgrass, *Zostera marina* (*3*). The bloom also devastated populations of *Argopecten irradians irradians* (bay scallop) and *Mytilus edulis* (blue mussel) by apparently causing starvation and total loss of larval recruitment (*4*, *5*). This resulted in severe monetary losses to local shellfishermen, especially the bay scallop industry in Peconic Bay, New York (*4*).

Massive "brown tide" blooms reappeared in Long Island bays in 1986 and, as in 1985, remained throughout the summer. Since then these blooms have recurred sporadically in isolated Long Island embayments, but have never returned to Narragansett Bay (*6*, *7*). Major environmental factors that have been found to contribute to these blooms include elevated salinities from drought conditions (*2*), elevation of organic compounds or micronutrients in bay waters from runoff (*7*, *8*), reduced grazing (*7*), and restricted circulation of bay waters (*9*).

Some cells observed during the original 1985 transmission electron microscope (TEM) survey from Rhode Island bloom water contained virus-like particles (VLPs) 130 to 150 nm in diameter, and the existence of a virus specific for *A. anophagefferens* was hypothesized to explain sudden decreases in algal populations (*1*). We therefore decided to isolate and cultivate these viruses.

At two Long Island bloom sites [West Neck Bay (WNB) in the Peconic Bay system and Blue Point (BP) in Great South Bay], a seawater sample of 20 liters was collected at the beginning of the bloom, on 6 July 1992 at WNB and on 7 July 1992 at BP. Sampling was repeated on 14 July 1992 at WNB. Each sample was filtered through a 0.2-μm Gelman Sciences capsule filter. The sample was then concentrated according to the methods of Suttle *et al.* (*10*), except that the lower filtration cutoff was 10,000 molecular weight (MW) instead of 30,000 MW to obtain a 200-ml concentrate that could contain potentially infective viruses. The ultrafiltrate (<10,000 MW) was also collected. Concentrate and ultrafiltrate were filter-sterilized through a 0.2-μm Nuclepore filter, transferred into sterile flasks, and stored at 5°C in the dark.

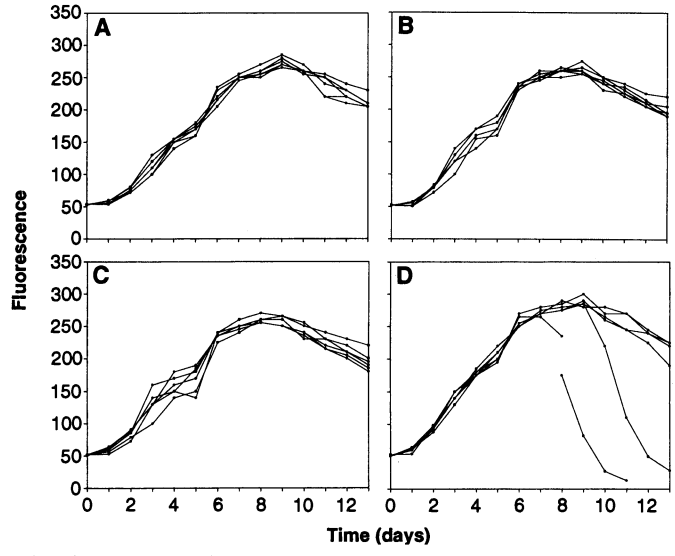

Fig. 1. Example of growth curves of *A. anophagefferens* in culture, testing for potential infectivity by viral concentrate from WNB 7/14/92 seawater. In each treatment, symbols (●) represent six replicate tubes, each containing 6 ml [some symbols overlap in (A), (B), (C), and (D)]. Treatment and control inocula were added on day 0 to healthy cultures. (**A**) Control, no virus added. (**B**) Control, 240 μl (4% of 6 ml) of microwaved (with inactivated viruses) concentrate added. (**C**) Control, 240 μl (4%) of ultrafiltrate (without viral particles), size fraction <10,000 MW added. (**D**) Example of experimental treatment, 360 μl (6%) of viral fraction concentrate added, with two infected replicates. Broken line represents inoculum extracted for serial transfer to healthy culture. Other experimental concentrations not shown were 120 μl (2%) and 240 μl (4%).

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Aureococcus anophagefferens was grown in Aquil media (*11*) to a cell concentration of approximately 4.5×10^5 cells per milliliter as determined by hemacytometer counts. The culture was divided among six replicate test tubes (6-ml volume) for each treatment. Treatments consisted of additions of various volumes of concentrate (viral fraction) and controls, to test for adverse effects from causes other than viral infection (Fig. 1).

Growth of *A. anophagefferens* in the tubes was monitored as in vivo fluorescence yield of cell chlorophyll a through the use of a Turner Designs fluorometer (Mountain View, California). When fluorescence reflected a sudden decrease of growth in experimental tubes relative to other replicates or to the control groups, a viral infection was suspected. Microscopic observations of infected cultures revealed only cellular debris and the absence of intact cells, indicating lysing of cells. Samples from lysed cultures were filter-sterilized through a 0.22-μm Millipore-GV filter, inoculated into fresh cultures of *A. anophagefferens*, and monitored for growth. Serial transfers of lysate were performed 10 times to amplify viruses that specifically attack *A. anophagefferens*.

There was a quantitative progression of infection in terms of percent viral concentrate from WNB 7/14/92. Two replicates of six showed infection in the 6% concentrate treatment (Fig. 1), only one replicate showed infection from the 4% concentrate, and no infection was observed from the 2% concentrate (*12*). Serial transfers from cultures that showed signs of lysis into healthy cultures consistently resulted in 100% reinfection (Fig. 2).

It was also possible to transfer the infection to large culture volumes of 600 ml with inoculum of 50 ml of lysate. Within 4 days after inoculation, the infected culture demonstrated a total loss of color, whereas there was continued growth in control cultures that had no inoculum added. Microscopic surveys again indicated cell lysis in the infected culture (*12*).

Samples from earlier bloom dates (WNB 7/6/92 and BP 7/7/92) showed infection in all concentrations (2 to 6%) and replicates of experimental treatments, suggesting that viral infectivity was greater at the peak of the bloom and demonstrating that viral activity was present in both the Great South Bay and Peconic Bay systems (*12*).

To evaluate whether isolated virus was DNA-based, we filtered samples through a 0.22-μm Millipore-GV filter and stained them with DNA-specific dye, 4-6-diamidino-2-phenylindole (DAPI), at a dilution of 0.1 ml of DAPI:1 ml of sample (*13, 14*). Virus particles appeared as pinpoints of blue under epifluorescence microscopy, indicating that the isolated virus contains DNA.

In electron microscope surveys of uninfected cultures, *A. anophagefferens* appeared as a small, round cell about 2.5 μm in diameter, similar to previous studies (*15*). Both uninfected and infected cultures were examined by transmission electron microscopy of uranyl acetate–stained samples on three separate occasions. In infected but not uninfected cultures, virus particles were found that resemble phages with a polygonal head approximately 50 to 70 nm (n = 25) in diameter and attached tail, 80 to 100 nm (n = 25; Fig. 3A). Culture exposed for only 10 min to viral inoculum showed tails of the virus particles attached to the cell with the heads pointed away (Fig. 3B). Culture exposed for approximately 45 min to viral inoculum showed no intact cells and some virus particles. The staining procedure resulted in clumping of cellular material, so that quantitative enumeration of viral particles was not possible.

The potential for other species to become infected by this virus was evaluated. Inocula of 360 μl of lysed culture of *A. anophagefferens* were filtered through 0.22-μm Millipore-GV filters and added to six replicate tubes for each unialgal species in exponential growth phase: *Thalassiosira weissflogii*, *Thalassiosira pseudonana*–clone 3H, *Nannochloris* sp., *Isochrysis* sp., and *A. anophagefferens*. Growth was monitored by in vivo fluorescence of cell chlorophyll a every 24 hours for a 2-week period. Of the species tested, *A. anophagefferens* was the only species in which cultures were lysed, indicating that the virus lysing *A. anophagefferens* would not infect the other species (*12*).

Only recently have viruses been described as having the potential to be significant factors in marine ecosystem dynamics (*16*). Virus-induced lysis was first established as a cause of mortality in microbial communities and is now being established as a significant factor in algal population control and as a contributor to dissolved organic levels in the marine system (*17*). Viral infections or the presence of VLPs have been identified in nine algal classes (*18*); however, most reports are incidental results of observations made during performance of general ultrastructural surveys of algal populations, and the virus was not isolated for cultivation and further study (*19*). Little is known about the significance of marine algal viruses in terms of their diversity, effectiveness as pathogens, and possible host ranges (*20*).

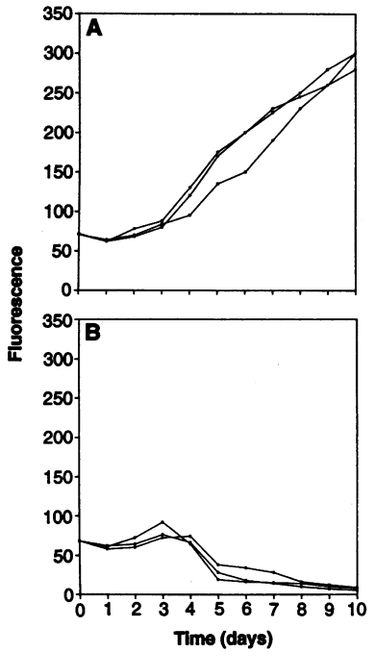

Fig. 2. Representative growth curves of cultures of *A. anophagefferens* after serial transfer of lysed cultures. Symbols (●) represent three replicate tubes, each containing 6 ml. Inocula were added on day 0 to healthy cultures. (**A**) Control, no lysate added. (**B**) Addition of 360 μl of inoculum from infected culture lysate from replicate in Fig. 1D.

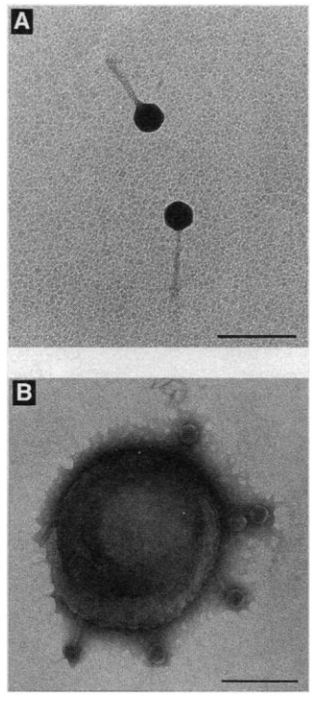

Fig. 3. Phage-like virus found in lysate and attached to *A. anophagefferens*. Samples were stained with 1% uranyl acetate and observed with TEM. (**A**) Example of free virus from lysed algal culture. Heads, 50 to 70 nm in diameter; tails, 80 to 100 nm in length (original magnification, ×60,000). Scale bar, 100 nm. (**B**) *Aureococcus anophagefferens* (cell size not representative because shrinkage occurred during staining) exposed to virus for 10 min (original magnification, ×40,000). Scale bar, 200 nm.

Although the phage-like appearance of the virus was surprising, marine viruses are highly diverse, and morphologies traditionally characteristic of phages cannot be assumed to infect only prokaryotes (*20*, *21*). Our experimental work indicates that the observed virus is the lysing agent of *A. anophagefferens*.

For *A. anophagefferens*, this study has established that viral control is an important consideration to be evaluated relative to the recurrence of blooms and their sudden dissipation. Viral lysis of *A. anophagefferens* during bloom periods may also release cellular products into the marine environment. This release would increase the concentration of dissolved and particulate organic matter (*22*) and possibly dimethylsulfide and acrylic acid (*23–26*), chemical compounds that might be associated with the chronic toxicity of bivalve starvation.

Another consideration in the population dynamics of *A. anophagefferens* is the inactivation of virus by environmental factors, thus allowing for blooms. For example, high concentrations of iron promote "brown tide" blooms in Long Island bays by stimulating growth of *A. anophagefferens* (*8*). Solid clay minerals and silts, iron oxides, and metal coagulants temporarily bind free virus particles (*27–29*), possibly precipitating and removing the potential for viral infection from the water column and effectively removing viral control on population densities.

REFERENCES AND NOTES

1. J. McN. Sieburth, P. W. Johnson, P. E. Hargraves, *J. Phycol.* **24**, 416 (1988).
2. E. M. Cosper *et al.*, in *Novel Phytoplankton Blooms*, E. M. Cosper, V. M. Bricelj, E. J. Carpenter, Eds. (Springer-Verlag, New York, 1989), pp. 317–340.
3. C. S. Yensch, D. A. Phinney, L. P. Shapiro, in (*2*), pp. 77–83.
4. V. M. Bricelj and S. H. Kuenstner, in (*2*), pp. 491–509.
5. G. A. Tracey *et al.*, *J. Shellfish Res.* **7**, 671 (1988).
6. T. J. Smayda and T. A. Villareal, in (*2*), pp. 159–187.
7. E. M. Cosper, C. Lee, E. J. Carpenter, in *Toxic Marine Phytoplankton*, E. Graneli, B. Sundstrm, L. Elder, D. M. Anderson, Eds. (Elsevier, New York, 1990), pp. 17–28.
8. E. M. Cosper, R. T. Garry, A. J. Milligan, M. H. Doall, in *Toxic Phytoplankton Blooms in the Seas*, T. J. Smayda and Y. Shimizu, Eds. (Elsevier, New York, 1993), pp. 667–673.
9. M. E. C. Vieira, E. M. Cosper, E. J. Beltrami, *ICES Mar. Sci. Symp.* **195**, 223 (1992).
10. C. A. Suttle, A. M. Chan, M. T. Cottrell, *Appl. Environ. Microbiol.* **57**, 721 (1991).
11. F. M. M. Morel, J. G. Rueter, D. M. Anderson, R. R. L. Guillard, *J. Phycol.* **15**, 135 (1979).
12. K. L. D. Milligan, unpublished data.
13. L. M. Proctor, thesis, University of New York at Stony Brook (1991).
14. K. G. Porter and Y. S. Feig, *Limnol. Oceanogr.* **25**, 943 (1980).
15. E. M. Cosper *et al.*, *Estuaries* **10**, 284 (1987).
16. L. M. Proctor and J. A. Fuhrman, *Nature* **343**, 60 (1990).
17. C. A. Suttle, A. M. Chan, M. T. Cottrell, *ibid.* **347**, 467 (1990).
18. E. L. Martin and R. Benson, in *Selected Papers in Phycology II*, J. R. Rosowski and B. C. Parker, Eds. (Phycological Society of America, Lawrence, KS, 1982), pp. 793–798.
19. J. A. Mayer and F. J. R. Taylor, *Nature* **281**, 299 (1979).
20. J. A. Fuhrman and C. A. Suttle, *Oceanography* **6**, 51 (1993).
21. J. L. Van Etten, L. C. Lane, R. H. Meints, *Microbiol. Rev.* **55**, 586 (1991).
22. L. M. Proctor and J. A. Fuhrman, *Mar. Ecol. Prog. Ser.* **69**, 133 (1991).
23. M. D. Keller, W. K. Bellows, R. R. L. Guillard, in (*7*), pp. 101–115.
24. A. Vairavamurthy, M. O. Andreae, R. L. Iverson, *Limnol. Oceanogr.* **36**, 59 (1985).
25. G. Tracey, R. Steele, L. Wright, in (*7*), pp. 233–237.
26. R. J. Charlson, J. E. Lovelock, M. O. Andreae, S. G. Warren, *Nature* **326**, 655 (1987).
27. R. B. Kapuscinski and R. Mitchell, *Water Res.* **14**, 363 (1980).
28. G. Bitton, *ibid.* **8**, 227 (1974).
29. E. Lund and V. Ronne, *ibid.* **7**, 863 (1973).

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p53 Status and the Efficacy of Cancer Therapy in Vivo

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The therapeutic responsiveness of genetically defined tumors expressing or devoid of the *p53* tumor suppressor gene was compared in immunocompromised mice. Tumors expressing the *p53* gene contained a high proportion of apoptotic cells and typically regressed after treatment with gamma radiation or adriamycin. In contrast, *p53*-deficient tumors treated with the same regimens continued to enlarge and contained few apoptotic cells. Acquired mutations in *p53* were associated with both treatment resistance and relapse in *p53*-expressing tumors. These results establish that defects in apoptosis, here caused by the inactivation of *p53*, can produce treatment-resistant tumors and suggest that *p53* status may be an important determinant of tumor response to therapy.

Although the tumor-specific action of most anticancer agents has been attributed to their debilitating effects on actively proliferating cells, an increasing body of evidence suggests that anticancer agents instead induce apoptosis [reviewed in (*1*, *2*)]. This view has profound implications for understanding the therapeutic response of human tumors. First, events subsequent to the interaction between anticancer agents and their primary intracellular targets may have a substantial effect on tumor response. Second, factors that increase the propensity for apoptosis may determine the therapeutic index whereby anticancer agents selectively destroy tumor cells. Finally, because apoptosis requires a genetic program, mutations in apoptotic pathways could produce drug-resistant tumors.

Studies suggest that the *p53* tumor suppressor gene is an essential component of the apoptotic program induced by anticancer agents in oncogenically transformed cells (*3*, *4*). To determine whether *p53* influences tumor responsiveness in vivo, we developed a transplantable fibrosarcoma model in which tumors differed primarily in their *p53* status. Embryonic fibroblasts transformed by coexpression of the adenovirus early region 1A (E1A) and activated *ras* oncogenes form tumors when subcutaneously injected into nude mice regardless of their *p53* status, but $p53^{+/+}$ cells are highly sensitive to apoptosis in vitro (*5*). In agreement with earlier studies, oncogenically transformed $p53^{+/+}$ cells formed fewer tumors and with a longer latency than $p53^{-/-}$ cells. The $p53^{-/-}$ cells produced tumors at all injected sites with an average latency of 8 ± 4 days, whereas the $p53^{+/+}$ cells produced tumors at $82 \pm 24\%$ ($P < 0.11$, t test) of sites injected, with an average latency of 18 ± 7 days ($P < 0.03$). After reaching a palpable size, tumors derived from both $p53^{+/+}$ and $p53^{-/-}$ cells grew at similar rates.

The *p53* status had a dramatic effect on tumor response to gamma irradiation. Most

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