

# Degree of ultraviolet radiation damage and repair capabilities are related to G+C content in marine vibriophages

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**ABSTRACT:** A key issue in the ecology of viruses in the marine environment is the rate of viral production and decay. The ultraviolet (UV) radiation in sunlight has been found to cause loss of infectivity in marine bacteriophages at rates nearly equal to all other decay mechanisms combined. There are 2 main host-mediated mechanisms that can repair UV-damaged phage DNA: photoreactivation and excision repair. Both these mechanisms were investigated in 2 marine *Vibrio parahaemolyticus* hosts as they catalyzed the reactivation of 7 phages. Photoreactivation was the dominant repair mode in all but one case. A significant correlation was found between G+C content of the phage DNAs (16 to 70%) and degree of DNA damage ( $r = 0.955$ ), indicating a strong relationship between the number of thymine dimer targets and the capability to photoreactivate DNA damage. Evolution of high G+C content may be a strategy for protection from UV damage in marine phages.

**KEY WORDS:** Vibriophages · Bacteriophages · G+C content · Ultraviolet radiation · DNA repair

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## INTRODUCTION

Marine viruses are now recognized to be the numerically dominant organisms in the world's oceans (Borshheim et al. 1990, Proctor & Fuhrman 1990, Suttle et al. 1990). Just over a decade has been spent investigating the ecological roles of these viruses in nutrient cycling (Bratbak et al. 1992, Wilhelm & Suttle 1999), food webs (Gonzalez & Suttle 1993), algal bloom mortality (Milligan & Cosper 1994, Nagasaki et al. 1994), and gene transfer (Jiang & Paul 1998). As a result of these studies, the contributions of marine viruses to biogeochemical cycles are beginning to be quantified and understood (Fuhrman 1999, Wommack & Colwell 2000). A key issue underlying all these concerns is the rate of viral production and decay. How quickly viruses are inactivated or removed from the system indicates how

much production must occur to maintain the steady state of  $10^9$  to  $10^{10}$  particles  $l^{-1}$ .

Murray & Jackson (1993) have made the only published attempt to model the effect of the interaction of ultraviolet (UV) light and mixing processes on marine virus survival. They predicted that the time of day the viruses were released from their host and how long the viruses spent circulating near the surface would heavily influence the dose of UV (and therefore the amount of damage) received by the viruses. The DNA damage caused by UV radiation in sunlight has been found to decrease infectivity in marine bacteriophages at rates equivalent to all other decay mechanisms (adsorption to particles, microbial interactions, flagellate grazing) combined (Suttle & Chen 1992). Further investigation comparing plaque counts of infectious phages to electron microscope counts of viral particles in the same microcosm showed that sunlight destroys phage infectivity more rapidly than viral particles (Wommack et al. 1996). Together, these results implied that the majority of viruses being counted in marine waters were not infective (Suttle & Chen 1992, Wommack et al. 1996).

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However, recent studies have shown that most of the viral particles remain infective, in spite of this solar damage (Wilhelm et al. 1998b). A model of viral production, including rates of particle destruction that are only 22 to 61 % of the rates of infectivity loss, indicates that some type of host-mediated repair must be restoring infectivity to the damaged viral DNA (Wilhelm et al. 1998b).

Solar radiation in the UVB wavelengths (290 to 320 nm) causes formation of cyclobutane pyrimidine dimers, while the UVA wavelengths (320 to 400 nm) cause single-strand breaks in DNA and DNA-protein crosslinks through photochemical interactions (Hartman et al. 1979, Casas-Fimet et al. 1984, Eisenstark 1987). There are 2 main host-mediated mechanisms that can repair UV-damaged phage DNA. The first, termed 'light repair' or photoreactivation, has been found to occur in all 3 kingdoms of life (Kiener et al. 1985, Sancar 1990, Friedberg et al. 1995). This is a single-step, light-dependent process catalyzed by the enzyme photolyase that specifically repairs pyrimidine dimers. The second repair system, a multi-enzyme complex, is known as nucleotide excision repair and responds to many types of DNA damage (Hanawalt et al. 1979, Lindahl 1982, Friedberg et al. 1995). Excision is not dependent on light and is sometimes called 'dark repair' to distinguish it from photoreactivation.

These repair mechanisms are now being studied in marine microbial systems, to determine the extent of UV DNA damage and repair in the ocean. Studies of marine bacterioplankton communities have revealed that substantial DNA repair is occurring each night (Jeffrey et al. 1996). The observation that expression of *recA*, one of the enzymes involved in excision repair, peaks just after sunset emphasizes the importance of dark repair in addition to whatever photoreactivation took place during the day (Jeffrey et al. 1996). Photoreactivation has been observed in culture experiments with marine *Vibrio natriegens* phage-host systems (Weinbauer et al. 1997, Wilhelm et al. 1998b), as well as in the total viral community (Weinbauer et al. 1997). DNA damage (number of pyrimidine dimers) was correlated to infectivity in a cultured marine phage, and these data were used to extrapolate to the proportion of the total marine viral community that is infective (>50 %) (Wilhelm et al. 1998b). This high level of infectivity in spite of a large degree of solar UV damage is attributed to repair by photolyase. In neither study (Weinbauer et al. 1997, Wilhelm et al. 1998b) was dark repair of the viruses quantified, but it appeared to play little if any role. It was suggested that this may be because photolyase is more efficient, as well as less energetically demanding (1 enzyme required rather than several), or that the amount of DNA damage was not great enough to induce the excision repair system (Weinbauer et al. 1997).

In the course of other experiments, we have found the phages of a marine *Vibrio parahaemolyticus* (strain [st.] 16) to be highly UV resistant, requiring over 500 J m<sup>-2</sup> of UVC to inactivate 99 % of the population. For comparative purposes, we obtained another marine *V. parahaemolyticus* (HER1165) and 4 of its phages from a culture collection. Our objective was to compare the contribution of photoreactivation and excision repair from each of these marine hosts and compare the repair between hosts and between individual vibriophages.

## MATERIALS AND METHODS

**Bacterial strains and viruses.** *Vibrio parahaemolyticus* st. 16 and its type phage 16 were isolated from surface waters at the St. Petersburg Pier in Tampa Bay, Florida, USA, in 1991 (Kellogg et al. 1995). *V. parahaemolyticus* st. 16 was also used as a host to isolate HAWI-5 from the Ala Wai Canal, Honolulu, Hawaii, USA, in 1993, and PEL8C-1 from a water sample taken at 1500 m in the Gulf of Mexico (Kellogg et al. 1995). All 3 16-like viruses appear to have identical morphologies, that of Bradley group A1, the Myoviridae. These 3 phages and their host bacterium have been deposited in the collection of the Félix d'Hérelle Reference Center for Bacterial Viruses (Ste-Foy, Quebec, Canada). Host *V. parahaemolyticus* HER1165 and its phages vp1, vp6, vp11 and vp12 were originally isolated off the coast of Japan (Koga et al. 1982) and were obtained from the Félix d'Hérelle Reference Center for Bacterial Viruses. These 4 viruses have been morphologically characterized in great detail (Ackermann et al. 1984). Vp1 is also a Myovirus, with morphotype A1, but is distinguished by neck appendages. The other 3 phages are Siphoviruses, but each is unique. Vp6 is morphotype B1, and its head is covered with spherical projections. Vp12 is also a B1, but with a large head and a single short tail fiber. Vp11 is morphotype B2, possessing a small elongated head.

**Lysate production.** Both bacterial hosts were grown in an artificial seawater media, ASWJP (Paul 1982) plus 5 g of peptone and 1 g yeast l<sup>-1</sup>, to exponential-phase growth in a shaking incubator at 30°C. The phages were propagated by top agar overlay, incubated overnight, eluted in 0.5 M Tris (pH 8) and 0.2 µm filtered as previously described (Kellogg et al. 1995).

**UV light source.** A 15 W UVC bulb was used, emitting primarily at 254 nm (Fisher Scientific). The bulb was mounted inside the top of a cardboard box measuring 48 cm (height) × 48 cm (width) × 30 cm (depth). The bulb's energy was measured over integrated time periods using an IL1400A Radiometer/Photometer (American Ultraviolet Company, Murray Hill, NJ, USA) with the SEL240/T2ACT3 detector (reading

range 190 to 400 nm) flush with the bottom surface of the box, facing the bulb. Three replicate readings were taken at each time of exposure (10 s to 3 min) and averaged. While UVC wavelengths are not present in the environment (they are filtered out by atmospheric ozone), they produce lesions identical to those caused by UVB (pyrimidine dimers); they are simply induced more efficiently, making this an acceptable model of environmental UV damage (Friedberg et al. 1995).

**UV irradiation of phages.** Suspensions of phage in 0.5 M Tris (pH 8) were aliquoted into sterile 100 × 15 mm petri dishes (4 ml each) and swirled so that the liquid formed a thin layer across the bottom of the plate. The phages were irradiated at room temperature (25°C) in a darkroom under a dim yellow safety light. The lid of the petri dish was removed, and dishes were placed inside the UV box described above for differing doses of energy. After removal from the box, 0.1 ml samples were taken from the lysate, serially diluted, and assayed for plaque-forming units (PFU) by top agar overlay (Adams 1959). The bacterial hosts were grown to exponential phase as in lysate production.

**Photoreactivation.** After performing top agar overlays in the darkroom, the plates were placed in a hood, 32 cm below a light bar containing two 40 W white fluorescent bulbs for 4 h (a dose of 0.6584 mol m<sup>-2</sup>). The plates were then removed to a dark 28°C incubator, allowed to grow overnight, and enumerated the next morning.

**Host cell reactivation (excision repair).** Irradiated phages were plated with unamended ASWJP+PY (peptone and yeast extract) plates and overlays to observe dark repair, or plated with ASWJP+PY amended with 1.5 mg ml<sup>-1</sup> caffeine (8 mM; Sigma) in the bottom agar, to block the excision repair pathway (Sauerbier 1964, Harm 1980). After overlaying in the darkroom, the plates were incubated at 28°C in total darkness.

**Base composition.** Viral DNA was extracted as described in the standard protocol for λ phage (Sambrook et al. 1989). The phage particles were precipitated with NaCl and polyethylene glycol (PEG), and then ultracentrifuged in a swinging bucket rotor to form a viral pellet. Proteinase K was used to remove the protein coat, followed by several phenol-chloroform extractions and ethanol precipitation of the viral DNA. The phage DNA was then digested with exonuclease III and P1 and run on a SmartSystem HPLC with MiniQ column (Pharmacia) under the following conditions: Buffer A: 20 mM Tris pH 8.2; Buffer B: Buffer A plus 1 M NaCl; 10% Buffer B after 10 min, 100% after 13 min; 240 μl min<sup>-1</sup> flow rate; simultaneous detection at 253 nm, 260 nm, and 274. The integrated area of the peaks was quantified after automatic baseline calculation (deoxy [d] GMP at 253 nm, dAMP at 260 nm, and both dCMP and dTMP at 274 nm).

**Statistical analysis.** Multiple comparison analysis and simple linear regression were done using the Statgraphics Plus software package (Manugistics, Rockville, Maryland, USA).

## RESULTS

### Maximum repair

We standardized the conditions for our experiments, irradiating the phages with UVC and then overlaying in a dark room under dim yellow light. For photoreactivation, the plates were exposed to fluorescent light for 4 h (a dose of 0.6584 mol m<sup>-2</sup>), which we had determined was the optimal dose for maximum photoreactivation (data not shown). Fig. 1 shows the survival curves of all 7 vibriophages, damaged by UVC and allowed both types of repair (photoreactivation and excision). The curves presented are averages of at least 2 replicate experiments. The top 3 curves that are almost overlapping are for 16, HAWI-5, and PEL8C-1, all of which share *Vibrio parahaemolyticus* st. 16 as their host. The next curve is vp6, followed by nearly identical curves for vp1 and vp11. The phage that showed the greatest UV sensitivity was vp12. To confirm that the differences in the curves were statistically significant, we log transformed the data to linearize the curves and

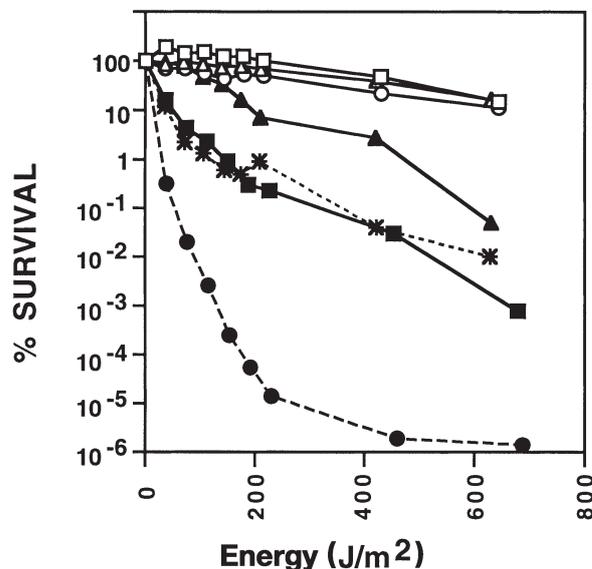


Fig. 1. Vibriophage survival curves with all repair mechanisms (photoreactivation and excision). 16 (□), HAWI-5 (Δ), PEL8C-1 (○), vp6 (▲), vp1 (■), vp11 (\*), vp12 (●). The 3 viruses coded with white symbols infect a common host, *Vibrio parahaemolyticus* st. 16. The 4 viruses coded with black symbols infect a different host, *Vibrio parahaemolyticus* HER1165

then performed Duncan's new multiple range test. The test found no significant differences between 16, HAWI-5, PEL8C-1, and vp6 or between vp1 and vp11. There were significant differences between each of these 2 groups and between each of the curves and vp12. Since production of these curves is very time and supplies consuming, we decided to continue our experiments with representatives of each group (16, vp6, vp1, and vp12). While vp6 technically falls into the first group, it has a different host from the other 3, and so was tested separately in the subsequent experiments.

### Dark repair

Repair was done in the dark to prevent photorepair. Statistical evaluation of these curves (done with Dun-

can's multiple range test, as the 'all repair' curves in Fig. 1) found no significant differences between the phages (data not shown). In all cases, excision repair alone resulted in a lower survival rate than when both photoreactivation and excision could function.

### Background

Survival curves were constructed from data with the 4 representative phages under conditions that blocked both photoreactivation and excision repair. The entire experiment was done in a darkroom under yellow safety light to prevent photoreactivation, and 1.5 mg ml<sup>-1</sup> (8 mM) caffeine was added to the media to inhibit excision repair. This concentration of caffeine blocks 95 to 99% of excision repair in *Escherichia coli* (Sauer-

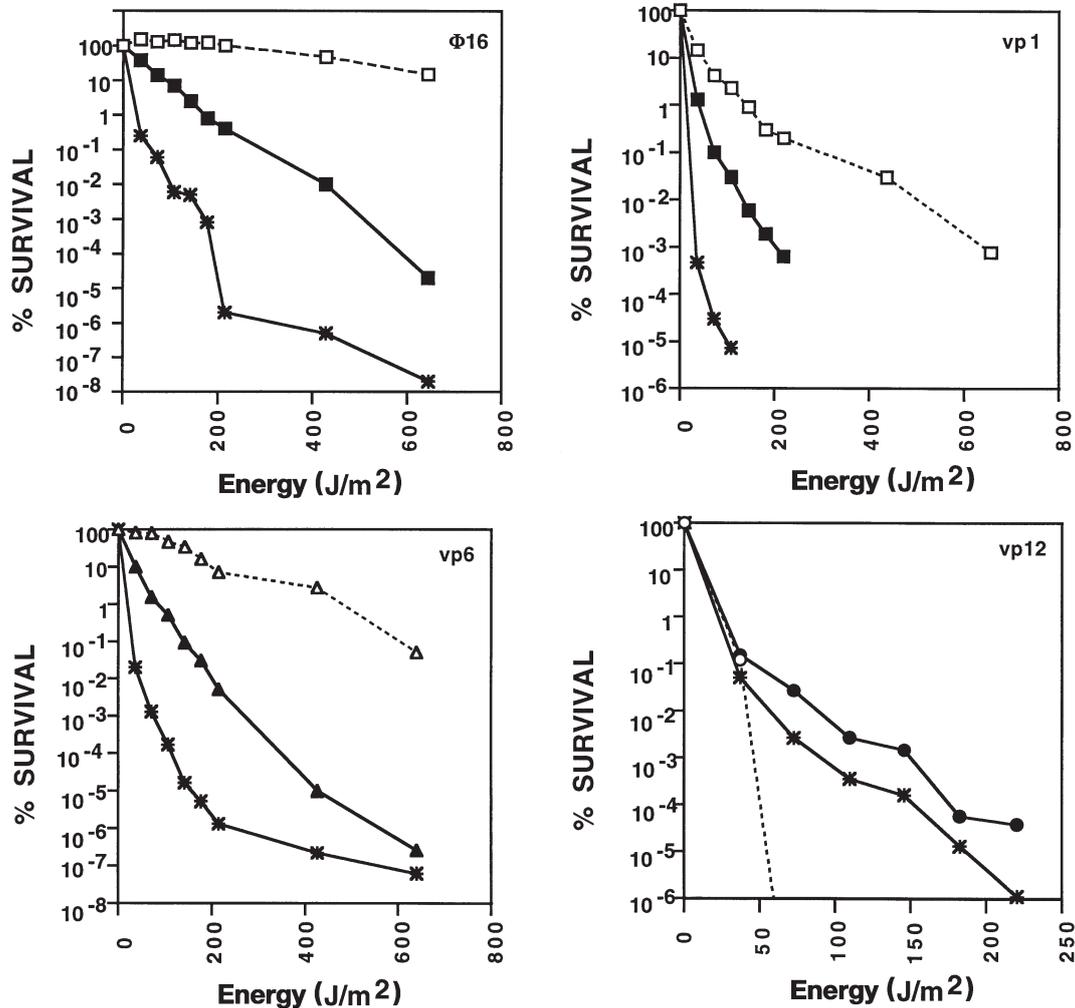


Fig. 2. Survival curves of 4 vibriophages comparing different repair mechanisms (calculated as explained in the text). Open (white) symbols indicate photoreactivation, closed (black) symbols indicate excision repair, and (\*) indicate background. Please note that the x-axis of vp12 has smaller increments than the other 3, and that the y-axes of the 2 right graphs stop at 10<sup>-6</sup> while the left 2 continue to 10<sup>-8</sup>.

bier 1964). However, it still permits growth of the bacterial hosts and plaque formation by both unirradiated (control) and irradiated phages (Harm 1980). The mechanism by which caffeine interferes with the excision pathway is still uncertain, but it is speculated to bind DNA, trapping excision repair proteins in unproductive DNA-protein complexes (Selby & Sancar 1990). All 4 vibriophages had very low survival when both repair mechanisms were blocked (curves with \* symbol in Fig. 2).

### Comparing repair mechanisms

To determine the importance of each repair mechanism for each vibriophage, we used the following equations:

$$\text{Photoreactivation} = \text{All Repair} - \text{Excision Repair} \quad (1)$$

$$\text{Excision Repair} = \text{Dark Repair} - \text{Background} \quad (2)$$

Fig. 2 provides a graphic comparison of the average survival curves for photoreactivation only, excision only, and background for each of the 4 representative phages. In the case of 16, the photoreactivation is so overwhelming that it approximates all repair, eclipsing excision repair by many orders of magnitude, and generating plaque counts >100% (higher than the unirradiated control plate). We believe that photoreactivation was so efficient during the 4 h light period that some repaired viruses were capable of additional rounds of infection and phage production. Excision repair plays a minor role for vp6 at lower energy (<142 J m<sup>-2</sup>), and at higher energies it is overtaken by photoreactivation. Both photoreactivation and dark repair are 5 orders of magnitude lower in vp1 than in 16. Excision makes a barely measurable contribution at low energies, and then what little photoreactivation occurs accounts for the majority of repair. The most interesting case is vp12, which has very little photoreactivation at the lowest energy, and then no detectable light repair at higher doses. Excision is approximating all repair, keeping survival about 1 order of magnitude higher than background.

### Base content

DNA from 6 of the vibriophages (16, HAWI-5, PEL8C-1, vp6, vp11, and vp12) was digested and then analyzed by HPLC to determine the base content of each phage's DNA (Table 1). The mass percentages of the 4 base nucleotides (A, C, T, G) add up to 100% in all cases except vp6 and vp12 (99%). The HPLC chromatograms showed only 4 peaks, so this is probably a

small quantification error and not an indication that there are additional modified nucleotides. However, the column used was not sensitive enough to detect minor modifications, such as methylation. The 3 phages infective for *Vibrio parahaemolyticus* st.16 (16, HAWI-5, and PEL8C-1) are already known, by *DpnI* digestion, to have N<sup>6</sup> methylation of some adenine residues (Kellogg et al. 1995). None of the HER1165 phages was digested by either *DpnI* or its isoschizomer *MboI* (which does not require methylation).

The range of G+C content was quite broad: 16 DNA was very GC rich (70%), as were HAWI-5 (60%) and PEL8C-1 (58%). Vp6 and vp11 were medial at 48 and 47%, respectively. Vp12 was extremely AT rich, having only 16% G+C (Table 1). All data points from the 'all repair' curves (Fig. 1) were log transformed to linearize the curves, and then linear regressions of those lines yielded slopes that serve as a rough estimate of survival under increasing UVC energy. A regression analysis of these slopes (UV inactivation coefficients) versus G+C content showed significant correlation ( $r = 0.955$ ; Fig. 3).

### DISCUSSION

Investigations of DNA repair of bacteriophages that infect various strains of *Vibrio cholerae* have determined that most hosts are capable of photoreactivation, but their ability to carry on excision repair is poor (Das et al. 1981, Palit et al. 1983). Sequencing of the *V. cholerae* genome has revealed an apparent frameshift in the *uvrB* gene (part of the excision pathway), which may impair its functionality (Jonathan Eisen pers. comm.). If poor excision is common in this genus, it could explain why photoreactivation is proportionally greater in all of the vibriophages we investigated except vp12 (Fig. 2). It might also explain the apparent lack of dark repair in the Weinbauer et al. (1997) and Wilhelm et al. (1998b) studies with *V. natriegens*.

It has also been observed that serologically and morphologically different phage display different

Table 1. Base composition of vibriophage DNAs. Numbers in columns indicate mass percent as determined by HPLC. d: deoxy

Phage	dCMP	dTMP	dAMP	dGMP	%G+C
φ16	34	10	20	36	70
φHAWI-5	29	14	26	31	60
φPEL8C-1	28	19	24	30	58
vp6	24	24	27	24	48
vp11	25	26	27	22	47
vp12	10	35	48	6	16

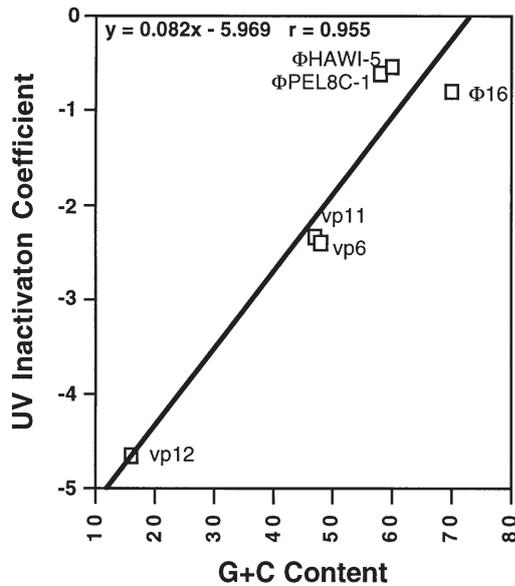


Fig. 3. Relationship between phage UV inactivation coefficients and G+C content. UV inactivation coefficient calculation is described in the text

levels of excision repair when reactivated by the same host: *Vibrio cholerae* (Palit et al. 1983), *Pseudomonas aeruginosa* (Kadavy et al. 2000). We also detected different levels of repair in different viruses (vp6, vp1, vp11, and vp12) that infect the same host (Fig. 1). However, the difference does not appear to be due to variable excision since the dark-repair-only curves were not significantly different among the phages, and the base line survival seen in the background curves was similar for all the vibriophages. This suggests that the variability could be due to some difference in efficiency of photoreactivation. It is also possible that the photoreactivation efficiency is identical but that the amount of damage incurred by the different viruses varied due to differences in the thickness of the protein capsids or the size and sequence content of the genomes.

The total repair potential (photoreactivation + excision repair) of both *Vibrio parahaemolyticus* hosts looks like it is roughly the same, since the vp6 'all repair' curve is not statistically significantly different from the 16/HAWI-5/PEL8C-1 curves (Fig. 1). The repair systems are present in the host, but the other 3 HER1165 phages (vp1, vp11, and vp12) are somehow unable to take advantage of them as effectively as vp6. This suggests that whatever is inhibiting or interfering with the repair must relate to the phages. Heavily modified bases (Harm 1980) or single-strand breaks in the phage DNA (Palit et al. 1983) can interfere with repair, but we did not find definitive evidence of any anomalous bases (uracil, hydroxymethyluracil, or N-thyminylputrescine) in the HER1165 vibriophages.

The role of light repair has been examined in marine viruses (Weinbauer et al. 1997, Wilhelm et al. 1998b) and has been touted as paramount for several reasons: (1) the mechanism is highly conserved (Friedberg et al. 1995), (2) the process is less energetically costly than excision repair (Friedberg et al. 1995), and (3) solar UVA damage may not induce the excision system (Turner & Eisenstark 1984). However, excision repair is important in that it is not just specific for thymine dimers (like the photolyase enzyme) but can repair other damage such as [6-4] photoproducts and some of the lesions caused by UVA. Excision repair is induced by solar UVB, not to mention that recA is constitutively produced at low levels, so this repair is occurring during the day as well as at night. We have found that in the absence of photoreactivation, excision repair can account for an increase in survival between 1 and 5 orders of magnitude greater than background (Fig. 2). How well this approximates what is occurring in the environment remains a topic of debate. Based on the argument that some lethal solar damage other than dimers (UVA-induced lesions) is less effectively dark repaired than 254 nm UV damage (Harm 1980) and some wavelengths of UVA inhibit excision repair (Turner & Eisenstark 1984), our methods could overestimate the amount of excision possible. Conversely, Kadavy et al. (2000) have found that starved or stationary-phase hosts (modeling bacteria in the environment) have increased dark repair, perhaps due to stress response. Their data suggest that experiments done with exponential-phase cells (like this study) would underestimate the amount of excision repair possible.

Kim & Sundin (2001) are developing a system of repair gene mutants in *Pseudomonas aeruginosa*, such that they disable the gene for photolyase (*phr*), one of the excision pathway genes (*uvrA*), or both. This approach will allow specific roles within light and dark repair to be better quantified. The technique is not yet applicable to a marine phage-host system, but it is very promising.

G+C contents in eubacteria range from 23% (*Mycoplasma*) to 75% (*Geodermatophilus*), which is greater than the range across the rest of the biological world (animals, plants, algae, protozoa, and archaea) (Neidhardt et al. 1990). Bacteriophage G+C contents are just as diverse as their hosts. Tailed phages are known to have G+C contents of 27 to 72%, usually close to that of the host (Francki et al. 1991). For example, *Vibrio cholerae* has 49% G+C (Schildkraut et al. 1962), and cholera phages range from 42 to 45% (Ackermann et al. 1984). Two of the HER1165 vibriophages (vp6 and vp11) fall just above this range (Table 1). The st. 16 vibriophages have considerably higher amounts, and Vp12 stands out as having an extremely low

concentration (16%). The only comparable G+C concentration known occurs in Entomopoxvirus B. This double-stranded (ds) DNA virus with a large genome (225 kb) has a G+C content of approximately 18.5% (Francki et al. 1991). This is the first report of vibriophages with both very high (70%) and very low (16%) G+C contents.

The strong correlation (0.95) we found between the inactivation coefficients of the viruses and their G+C content suggests that having an AT-rich genome, with its higher potential for dimer (T-T) sites, leads to a greater potential for UV damage. Additionally, a recent study suggests that AT-rich DNA can contribute to UV damage by enhancing the generation of reactive oxygen species (superoxide anion, hydrogen peroxide, and molecular singlet oxygen), which cause oxidative damage (Wei et al. 1998).

A wide range of decay rates has been calculated for both individual and populations of phage from the marine environment—0.7% h<sup>-1</sup> (Wilhelm et al. 1998a) to 80% h<sup>-1</sup> (Suttle & Chen 1992)—with quite a bit of variation in between (see Wommack & Colwell 2000 for a more exhaustive listing). These divergent rates may be due in part to differences in experimental approach: artificial seawater microcosms versus genuine seawater; absorbance of UV by plastic sample bags or glass lids, etc. However, it is likely that a larger portion of the variability is due to differences in the viruses' sensitivity to UV, which in turn could be due to their G+C content. There is already some evidence that marine viruses may have an evolutionary adaptation to local sunlight levels (Noble & Fuhrman 1997). The st. 16 vibriophages were isolated from tropical environments, while the HER1165 phages were isolated in temperate waters, so it is possible that the higher G+C content observed in the st. 16 viruses is an evolutionary adaptation to combat high UV exposure.

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#### LITERATURE CITED

- Ackermann HW, Kasatiya SS, Kawata T, Koga T and 5 others (1984) Classification of *Vibrio* bacteriophages. *Intervirology* 22:61–71
- Adams MH (1959) *Bacteriophages*. Wiley-Interscience, New York
- Borsheim KY, Bratbak G, Heldal M (1990) Enumeration and biomass estimation of planktonic bacteria and viruses by transmission electron microscopy. *Appl Environ Microbiol* 56:352–356
- Bratbak G, Heldal M, Thingstad F, Riemann B, Haslund OH (1992) Incorporation of viruses into the budget of microbial C-transfer: a first approach. *Mar Ecol Prog Ser* 83:273–280
- Casas-Fimet JR, Toulme J, Cazenave C, Santus R (1984) Role of tryptophan and cysteine in binding of gene 32 protein from phage T4 to single-strand DNA. Modification of crucial residues by oxidation with selective free free-radical anions. *Biochemistry* 23:1208–1213
- Das G, Sil K, Das J (1981) Repair of ultraviolet light induced DNA damage in *Vibrio cholerae*. *Biochim Biophys Acta* 655:413–420
- Eisenstark A (1987) Mutagenic and lethal effects of near-ultraviolet radiation (290–400 nm) on bacteria and phage. *Environ Mol Mutag* 10:317–337
- Francki RIB, Fauquet CM, Knudson DL, Brown F (1991) Classification and nomenclature of viruses: fifth report of the International Committee on Taxonomy of Viruses. Springer-Verlag, New York
- Friedberg EC, Walker GC, Siede W (1995) DNA repair and mutagenesis. ASM Press, Washington, DC
- Fuhrman JA (1999) Marine viruses and their biogeochemical and ecological effects. *Nature* 399:541–548
- Gonzalez JM, Suttle CA (1993) Grazing by marine nanoflagellates on viruses and virus-sized particles: ingestion and digestion. *Mar Ecol Prog Ser* 94:1–10
- Hanawalt PC, Cooper PK, Ganesan AK, Smith CA (1979) DNA repair in bacteria and mammalian cells. *Annu Rev Biochem* 48:783–836
- Harm W (1980) Biological effects of ultraviolet radiation. Cambridge University Press, Cambridge
- Hartman PS, Eisenstark A, Pauw PG (1979) Near-ultraviolet radiation plus H<sub>2</sub>O<sub>2</sub> inactivation on phage T7: DNA-protein crosslinks prevent DNA injection. *Proc Natl Acad Sci USA* 76:3228–3232
- Jeffrey WH, Pledger RJ, Aas P, Hager S and 3 others (1996) Diel and depth profiles of DNA photodamage in bacterioplankton exposed to ambient solar ultraviolet radiation. *Mar Ecol Prog Ser* 137:283–291
- Jiang SC, Paul JH (1998) Gene transfer by transduction in the marine environment. *Appl Environ Microbiol* 64:2780–2787
- Kadavy DR, Shaffer JJ, Lott SE, Wolf TA and 5 others (2000) Influence of infected cell growth state on bacteriophage reactivation levels. *Appl Environ Microbiol* 66:5206–5212
- Kellogg CA, Rose JB, Jiang SC, Thurmond JM, Paul JH (1995) Genetic diversity of related vibriophages isolated from marine environments around Florida and Hawaii, USA. *Mar Ecol Prog Ser* 120:89–98
- Kiener A, Gall R, Rechsteiner T, Leisinger T (1985) Photoreactivation in *Methanobacterium thermoautotrophicum*. *Arch Microbiol* 143:147–150
- Kim JJ, Sundin GW (2001) Construction and analysis of photolyase mutants of *Pseudomonas aeruginosa* and *Pseudomonas syringae*: contribution of photoreactivation, nucleotide excision repair, and mutagenic DNA repair to cell survival and mutability following exposure to UV-B radiation. *Appl Environ Microbiol* 67:1405–1411
- Koga T, Toyoshima S, Kawata T (1982) Morphological varieties and host ranges of *Vibrio parahaemolyticus* bacteriophages isolated from seawater. *Appl Environ Microbiol* 44:466–470
- Lindahl T (1982) DNA repair enzymes. *Annu Rev Biochem* 51:61–87
- Milligan KLD, Cospér EM (1994) Isolation of virus capable of lysing the brown tide microalga *Aureococcus anophagefferens*. *Science* 266:805–807
- Murray AG, Jackson GA (1993) Viral dynamics II: a model of the interaction of ultraviolet light and mixing processes on

- virus survival in seawater. *Mar Ecol Prog Ser* 102:105–114
- Nagasaki K, Ando M, Imai I, Itakura S, Ishida Y (1994) Virus-like particles in *Heterosigma akashiwo* (Raphidophyceae): a possible red tide disintegration mechanism. *Mar Biol* 119:307–312
- Neidhardt FC, Ingraham JL, Schaechter M (1990) Physiology of the bacterial cell: a molecular approach. Sinauer Associates, Inc, Sunderland
- Noble RT, Fuhrman JA (1997) Virus decay and its causes in coastal waters. *Appl Environ Microbiol* 63:77–83
- Palit BN, Das G, Das J (1983) Repair of ultraviolet light-induced DNA damage in cholera bacteriophages. *J Gen Virol* 64:1749–1755
- Paul JH (1982) Use of Hoechst dyes 33258 and 33342 for enumeration of attached and planktonic bacteria. *Appl Environ Microbiol* 43:939–944
- Proctor LM, Fuhrman JA (1990) Viral mortality of marine bacteria and cyanobacteria. *Nature* 343:60–62
- Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
- Sancar G (1990) DNA photolyases: physical properties, action mechanism, and roles in dark repair. *Mutat Res* 236:147–160
- Sauerbier W (1964) Inhibition of host cell reactivation in phage T1 by caffeine. *Biochem Biophys Res Commun* 14:340–346
- Schildkraut CL, Marmur J, Doty P (1962) Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. *J Mol Biol* 4:430–443
- Selby CP, Sancar A (1990) Molecular mechanisms of DNA repair inhibition by caffeine. *Proc Natl Acad Sci USA* 87:3522–3525
- Suttle CA, Chen F (1992) Mechanisms and rates of decay of marine viruses in seawater. *Appl Environ Microbiol* 58:3721–3729
- Suttle CA, Chan AM, Cottrell MT (1990) Infection of phytoplankton by viruses and reduction of primary productivity. *Nature* 347:467–469
- Turner MA, Eisenstark A (1984) Near-ultraviolet radiation blocks SOS responses to DNA damage in *Escherichia coli*. *Mol Gen Genet* 193:33–37
- Wei H, Ca Q, Rahn R, Zhang X, Wang Y, Lebwohl M (1998) DNA structural integrity and base composition affect ultraviolet light-induced oxidative DNA damage. *Biochemistry* 37:6485–6490
- Weinbauer MG, Wilhelm SW, Suttle CA, Garza DR (1997) Photoreactivation compensates for UV damage and restores infectivity to natural marine virus communities. *Appl Environ Microbiol* 63:2200–2205
- Wilhelm SW, Suttle CA (1999) Viruses and nutrient cycles in the sea: viruses play critical roles in the structure and function of aquatic food webs. *BioScience* 49:781–788
- Wilhelm SW, Weinbauer MG, Suttle CA, Jeffrey WH (1998a) The role of sunlight in the removal and repair of viruses in the sea. *Limnol Oceanogr* 43:586–592
- Wilhelm SW, Weinbauer MG, Suttle CA, Pledger RJ, Mitchell DL (1998b) Measurements of DNA damage and photoreactivation imply that most viruses in marine surface waters are infective. *Aquat Microb Ecol* 14:215–222
- Wommack KE, Colwell RR (2000) Virioplankton: viruses in aquatic ecosystems. *Microbiol Mol Biol Rev* 64:69–114
- Wommack KE, Hill RT, Muller TA, Colwell RR (1996) Effects of sunlight on bacteriophage viability and structure. *Appl Environ Microbiol* 62:1336–1341

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