

# The biochemistry of the undead

# Silent killers

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The Earth's oceans are teeming with life. Despite our obsession with the terrestrial environment we inhabit, it is the oceans that first gave rise to life 2.9 billion years ago, and, with an extra billion years or so of evolution behind them, it is the oceans that harbour the greatest genetic and metabolic diversity on the planet. Yet we know surprisingly little about the biochemistry of our oceans. Microbial life forms covering the three cellular kingdoms (Archaea, Bacteria and Eukarya) represent by far the largest fraction of the total biomass existent in the oceans. However, a poorly studied and, until recently, largely ignored other kingdom also exists: the viral kingdom. Entirely dependent on the three cellular kingdoms of life, the viral kingdom rules above with an iron fist, ruthlessly and mercilessly destroying their hosts in order to replicate and produce their own progeny.

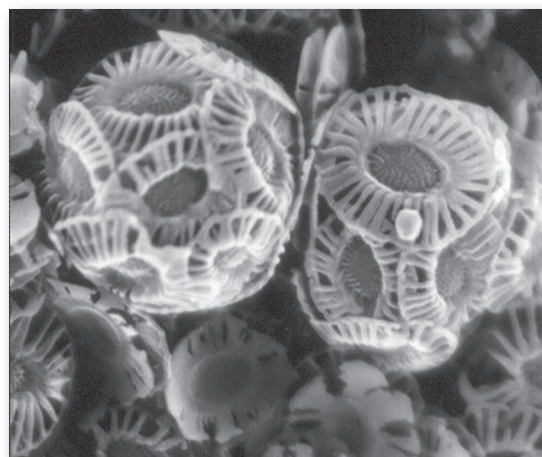
It was only a few decades ago that the impact of this relentless killing activity was begun to be appreciated. This dawning realization was triggered by the discovery of an immense viral abundance in the oceans. Numbering in excess of 10 million per ml of water (an estimated total of  $10^{31}$  viruses in the oceans)<sup>1</sup>, it was soon realized that the marine viruses were interacting with their cellular counterparts in the microbial kingdom on an unprecedented scale. With an estimated  $10^{23}$  new infections occurring every second within the ocean, viruses are estimated to kill up to 40% of marine bacteria on a daily basis<sup>2</sup>. This lytic function is of fundamental importance to global biogeochemical cycling and ecosystem function, which would undoubtedly collapse without the constant 'lubrication' of the planets biological machinery.

Crucially, the impact of viral infection, when it is given consideration, is often studied from the end point: models are often interested only in the loss of cells and the cycling of nutrients. However, the journey may be just as important as the destination. With viral infection occurring on such a phenomenal scale, a significant proportion of microbial cells in the marine environment are infected at any given time. While on the surface these cells may look and act alive as they play host to the selfish replicators within, in reality these cells have been handed a life sentence and are merely shadows of their former selves. **They can be regarded as the ocean's undead.** These hapless host vessels are either lost entirely to the viral underworld or are in vicious intracellular battle for survival, and through their sheer abundance, contribute significantly to the biochemistry of the oceans. When the battle has been lost, zombified cells mix alongside their healthy counterparts with their metabolic activities hijacked by the enemies within their walls as they move blindly through the motions of producing as many

new viruses as possible until decay and rot sets in, leading to the disruption of cellular integrity.

## Softly, softly, catchee algae

At the very bottom of the marine food chain, the phytoplankton, a large group of genetically diverse oxygen-generating photosynthetic organisms (including cyanobacteria, microalgae and seaweeds) fix  $\text{CO}_2$  and convert it into organic compounds such as lipids, sugars and proteins. Their importance cannot be underestimated: they are responsible for around half of the oxygen we breathe. They are quiet, unassuming, mostly single-celled workers that ensure that life on Earth can exist. The abundance of viruses in the water column ensures that viral infection has a massive impact on their community dynamics. When algal numbers are relatively low, all viruses can do is float about in the water column



Coccolithophores plus virus attached to lith

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waiting to come into contact with a suitable host. However, algae are highly opportunistic and, when conditions are right (for example when a new source of nutrients becomes available, or when light and temperature becomes more favourable), phytoplankton species can swiftly overtake populations and dominate. During these so-called bloom events, a single algal species can reach concentrations of over a billion cells per litre. Harmful red tides of toxic dinoflagellates are a well-known example of such community takeovers. In addition to causing water discoloration, the neurotoxin saxitoxin (a sodium channel blocker) is often produced in large amounts under such conditions and can decimate fisheries and cause paralytic shellfish poisoning in humans.

Yet, algal blooms also play into the hands of viruses, by producing large concentrated populations of relatively homogenous potential host populations. No longer reliant on long periods spent in limbo between infections as they wait for contact with a suitable host, algal blooms create a situation ripe for rapid viral propagation. Viruses have been found to be one of the major causes of bloom demise. As ever, population breeds disease. Although slow, relentless background infections will always occur within communities and account for the bulk of infections, for some lucky viruses their host will effectively come looking for them under bloom conditions.

### Cliffs, clouds and wartime songs

Perhaps the most famous of the phytoplankton, the coccolithophore *Emiliana huxleyi* has global distribution and is well known for forming both coastal and open ocean blooms in temperate latitudes than can cover up to 100 000 km<sup>2</sup> <sup>3</sup>. As the most abundant and ubiquitous coccolithophore in modern oceans, it has become a key species for studies on global biogeochemical cycles and climate modelling<sup>4</sup>. In a world obsessed with carbon footprints and emissions, *E. huxleyi* stands out as an organism capable of both fixing CO<sub>2</sub>, and of sequestering carbon in an inorganic form during the construction of its elegant chalk outer scales. Measuring just a few micrometres in diameter, but growing to high concentrations during times of blooming, over geological time, the release of its liths to the ocean floor is capable of forming structures such as the White Cliffs of Dover. Amazingly, the death of these algae can even influence the weather. When dimethylsulfoniopropionate (DMSP), which is used as an intracellular osmolyte, is released following cellular disintegration, it is rapidly cleaved to produce dimethylsulfide which rises up to the atmosphere, becomes oxidized and forms cloud condensation nuclei<sup>5</sup>.

Viruses, of course, will help to accelerate these processes. Given the attention lavished upon *E. huxleyi*, it is unsurprising that coccolithoviruses have been isolated during *E. huxleyi* blooms. Indeed, the classical milky waters produced towards the end of *E. huxleyi* blooms are not full of healthy *E. huxleyi* cells at all; they comprise mostly dead and infected dying cells that have released their liths into the surrounding waters. When sampled, these milky waters are usually full of coccolithoviruses. Dame Vera Lynn may well have found herself short of material during the Second World War if the action of the coccolithoviruses over millions of years had not helped to destroy blooming coccolithophore populations to create the chalk cliffs her bluebirds were to famously fly over.

### Tiny giants

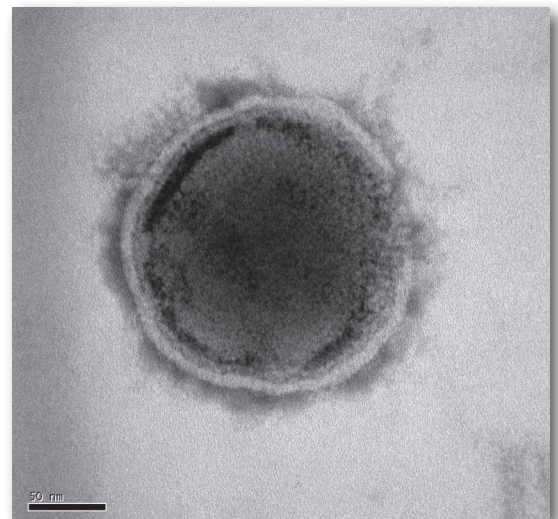
You can see how easy it is to fall into the trap of focusing on the elegant end products (the weather effects and the geological structures) of coccolithophore/coccolithovirus infection. However, perhaps even greater beauty can be found in the metabolic intricacies that have been revealed by studying the infection process at the molecular level. The biochemistry of the infected cells, the undead, in the case of *E. huxleyi* is truly remarkable. Indeed, the coccolithoviruses have been integral to changing the way we viewed viruses. With gigantic genomes in excess of 400 000 bp, these double-stranded DNA viruses contain almost 500 genes and, with such complexity, must have the potential to manipulate



The White Cliffs of Dover



*E. huxleyi* bloom from space



The coccolithovirus EhV-86



Induced *E. huxleyi* bloom in Norwegian fjord

and coerce their hosts in subtle yet complex and probably highly ruthless ways.

The coccolithovirus genome<sup>6</sup>, published shortly after its more famous cousin, the mimivirus<sup>7</sup> (currently the world's largest sequenced virus, with a genome of over a million bases, larger even than that of some free living cells) has helped to reinvigorate the debate on the evolution and the status of viruses. The term 'girus' (for giant-virus) was coined shortly after and has added to the debate as to whether some (and maybe all) viruses should actually be regarded as alive. Regardless of the outcome of such debates, the coccolithovirus genome has revealed some remarkable features. Our eyes have been opened to a new degree of infection where viruses have the potential to control and manipulate cellular metabolic pathways to a higher level than previously observed. Perhaps the most startling observation that can be made about the coccolithovirus genome is its genetic novelty: the vast majority of genes (>80%) are

of unknown function and contain few database matches. The usual suspects are also there, of course, providing the crucial 'classical' virus associated functions such as nucleotide metabolism (RNA/DNA), protein metabolism and structural protein assembly. However, in comparison with other related algal viruses (known as phycodnaviruses), a mere dozen genes can be found in common at most<sup>8</sup>. This leaves a startling amount of novelty remaining, which has, presumably, been acquired, selected for and evolved since the coccolithovirus lineage developed, and has become integral to its infection strategy.

### The haunting: sphingolipid pathway

Not all of the coccolithovirus novelty is of unknown function. A surprising feature of coccolithoviruses is the presence of an almost complete pathway for sphingolipid production. Sphingolipids are membrane lipids implicated

in the formation of lipid rafts, the regulation of a myriad of signalling pathways and even control of eukaryotic programmed cell death. For years, the presence of this pathway on coccolithovirus genomes was a complete mystery: now it is only *mostly* a mystery. The sequencing of the *E. huxleyi* genome quickly revealed that the viruses had stolen the sphingolipid pathway genes directly from their host<sup>9</sup>. Transcriptional profiling revealed their expression is crucial to the infection process, and that during the early stages of infection a metabolic war is waged between virus and host, the winner, in some part, determined by who can control sphingolipid metabolism<sup>10</sup>. In a cruel twist of fate, it appears as though the coccolithovirus uses its hosts own biosynthetic pathway against itself. Each time *E. huxleyi* becomes infected, the ghostly spectre of its own ancestral metabolic pathway returns to haunt it, taunting it and manipulating the cellular biochemical composition for its own selfish needs. In addition to spawning and contributing to good humoured theories such as *The Survival of the Fattest* and *The Cheshire Cat Hypothesis* (whereby sphingolipids are used as a signal to control sexual cycle and escape future infection<sup>11</sup>), the viral sphingolipid pathway has intrigued researchers the world over.

## The (unknown) nature of the beasts

While the sphingolipid pathway steals the headlines, it accounts for just six of the genes contained on coccolithovirus genomes. A plethora remains. In addition, putative hydrolase, esterase, lipase, proteases, kinases, phosphatases, nucleases, helicases, thioredoxins and ligase function can also be predicted along with many DNA-binding proteins which presumably have complex regulatory functions over host and/or virus genome activity during infection. Yet, the function of the vast majority of the genome remains a complete mystery. An entire 100 kb section fails to contain even one functionally annotated gene. During infection, the potential impact that its gene products have is difficult to ignore: they are the most highly expressed of all the genes during the early stages of infection<sup>12</sup>.

Yet this is far from unusual for algal viruses and other marine viruses: the majority of genes are novel and we have little insight into their functional relevance. Occasionally

we are provided with a glimpse into the elegant metabolic manipulations that go on during infection, the coccolithovirus sphingolipid pathway is an excellent example. Other examples exist such as the cyanophage-encoded photosynthetic machinery. During the midst of viral infection, when host cells exist in their viral-induced undead state blindly clinging on to cellular integrity, their zombified metabolic function remains both largely ignored and uncharacterized. Yet, as we showed above, the scale of infection ensures that, despite our ignorance, the biochemistry associated will contribute a significant proportion to global metabolic function and biochemical activity. Through the coccolithoviruses, we have seen a sparkle in the eyes of the zombie. All may be lost for these poor hijacked vessels, but their contribution to global function is only now being found. ■

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