



Origins of DEATH

Programmed cell death is usually seen as the unique prerogative of plants and animals. So how is it that photosynthetic plankton have been killing themselves by uncannily similar methods for billions of years? **Nick Lane** investigates.

One evening 20 years ago, Paul Falkowski left the lab so tired that he omitted to refresh the solution in his culture flasks of *Emiliania huxleyi*, one of the world's most widespread coccolithophores. The following morning he was shocked to find the flasks full of clear solution, the merest sediment lining the bottom, all that remained of the plankton. "I had never seen anything like it," he recalls. "They just dissolved overnight."

The speed and totality of their demise couldn't be put down to run-of-the-mill mortality, which leaves a gory mess of living cells, dying cells and clumps of dead matter. The tidy dissolution of the plankton was more reminiscent of apoptosis in animals — a synchronized wave of death orchestrated by some invisible hand to some unknowable end. Falkowski became determined to reveal that hand and understand its purpose, and now, in his lab and others, the revelations are beginning to add up.

Now a professor of marine biogeochemistry at Rutgers University in New Jersey, Falkowski is better known for his research on marine productivity and nutrient cycles. From that mainstream perspective, his interest in programmed cell death among plankton might seem a quirky morbid streak.

Dying, though, is something phytoplankton

and eukaryotes that photosynthesize, such as coccolithophores and diatoms — fix as much carbon every year as all the plants on all the continents. Yet at any one time they account for just 1% of Earth's biomass. This means their rate of turnover is huge; on average, the world's phytoplankton population is replaced once a week.

Most models of marine systems simply put that mortality down to bad luck. They tacitly assume that phytoplankton are in principle immortal, but in practice always seem to be eaten by zooplankton, wiped out by viral infections or starved by nutrient deprivation. But Falkowski thinks that there is more to it than that. Phytoplankton don't just dissolve when neglected in the lab. Vast marine blooms, too, can disappear overnight. If that process is indeed a manifestation of programmed cell death, then it has implications that not only touch on the global nutrient cycling, but also foreshadow the ultimate causes of our own mortality. The phytoplankton of the ancient oceans might have been some of the first creatures to learn how to die.

Falkowski's hunch that the dissolution of his phytoplankton might have parallels with

be remarkably close to the mark. Apoptosis is masterminded with extraordinary finesse by a group of protein-splitting enzymes known as caspases. The scissor-like ability to cut proteins that sets these enzymes apart is constrained to very particular sequences of amino acids. They operate in cascades in which one caspase activates the next by slicing through a protective protein sheath. Each step amplifies the death signal until an army of executioners has been let loose on the cell, dismantling membrane structures, slicing up DNA, dicing proteins.

This carefully orchestrated cascade leaves tell-tale signs at the microscopic level. The nuclear material condenses; chromosomes fragment; the cell membrane blebs off into tiny bubbles; internal organelles disintegrate; then the cell dissolves around them.

Working with Kay Bidle, a marine microbiologist also at Rutgers, Falkowski found that cultured phytoplankton precisely reproduced every microscopic step. The pair also found that antibodies raised against human caspases cross-reacted with extracts taken from phytoplankton, suggesting that the extracts must contain proteins similar to the caspases¹.

"Much is owed to a lucky choice of bacteria."
— Eugene Koonin

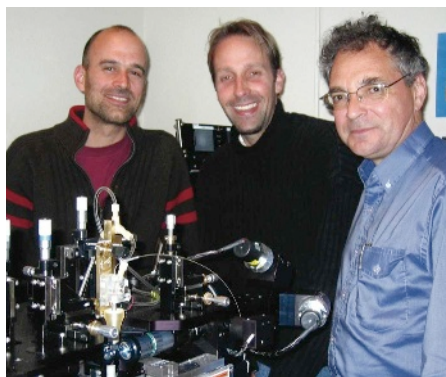
inhibitors of caspases interfered with the cells' death programme. Remarkably, the evidence of programmed death was not constrained to eukaryotes; it was found in photosynthetic cyanobacteria too.

By the late 1990s, similar findings had been reported in plants. But no one had managed to identify the caspases presumed responsible, either in the plants or in the plankton. Then in 2000, researchers at the National Center for Biotechnology Information in Bethesda, Maryland, and the biotechnology firm Genentech in San Francisco, California, discovered a related family of proteins by analysing genes from a wide range of species for sequences that might produce caspase-like activity when expressed². The *in silico* discovery of these 'metacaspases' paved the way to understanding programmed cell death in plants, algae and fungi, and offered the first real insight into the origin of the caspases, now known to be found only in animals.

Cosmopolitan lifestyle

Within a couple of years, Eugene Koonin and Lakshminarayanan Aravind, two of the researchers on the original discovery team, had sketched out the protein family's distribution across the tree of life³. In doing so, they confirmed the molecules' presence within a few groups of bacteria that show a touch more complexity than most of their peers, notably the photosynthetic cyanobacteria, which sometimes form multicellular chains. It was already clear, Koonin recalls, that the metacaspases and caspases derived from the same ancestor. Identifying that ancestor, though, is hard. Bacteria have very fluid chromosomes, and frequently incorporate genes derived from totally unrelated bacteria. So although cyanobacteria have the richest metacaspase reservoir, and thus might seem a likely point of origin, two other groups of bacteria have metacaspase genes and so need to be considered: the actinomycetes (a group of soil bacteria) and a few of the α -proteobacteria.

The most compelling account of the origin of mitochondria, the 'power plants' of almost all modern eukaryotic cells, is that they are descended from the α -proteobacteria. Metacaspases are widespread among eukaryotes, so they were probably acquired at an early point in eukaryotic history. "The fact that some α -proteobacteria contain metacaspases makes mitochondria the most likely source of ancestral metacaspases in eukaryotic cells," Koonin says. How the α -proteobacteria got their metacaspases, though, is an open question, and



Sorting out cells: Assaf Vardi (left), Kay Bidle (centre) and Paul Falkowski are trying to decipher why phytoplankton might want to kill themselves.

It is also possible that some eukaryotes received their metacaspases from two different lineages — once via the α -proteobacteria that became the mitochondria, and once via the engulfed cyanobacteria that evolved into chloroplasts. Bidle points out that some of the metacaspases found only in algae and plants are aimed at targets in chloroplasts, which hints they may have come in that way.

But why do cyanobacteria have all those metacaspases? In their 2002 paper³, Koonin and Aravind argued that, in general, bacterial metacaspases have some sort of signalling role; but they also wondered whether, in a few more-complex 'cosmopolitan' bacteria, they might mediate cell death as the animals' caspases do. And that was exactly what Bidle and Falkowski were wondering, too.

Metacaspases are in at least one sense not caspases: for one thing few, perhaps even none, cleave the classic caspase target sequence. For this and other reasons some researchers, notably Frank Van Breusegem and his colleagues at Ghent University in Belgium, doubt that metacaspases execute cell death in plants and phytoplankton — or at least whether the case is yet properly proved.

Bidle and Ilana Berman-Frank, at Bar Ilan University in Israel, have put some effort into that proof. They have shown that the number of metacaspases rises and that caspase activity sky-rockets during cell death in cyanobacteria such as *Trichodesmium* — a subtropical bloom-forming bacterium that might have evolved as long as three billion years ago. The microscopic demise of these cells looks like apoptosis — and the cyanobacteria in question turn out also to make several other key enzymes found in the cell-death cascade of animals. (Apop-

NACHT-family NTPases, since you ask.)

But this evidence is circumstantial, and the same team has also found a lot of metacaspases that are not involved in cell death. This February, for example, Bidle and his colleagues reported that *Thalassiosira pseudonana*, a single-celled diatom, has six putative metacaspases⁴, not far short of the nine found in plants such as the thale cress *Arabidopsis*, or indeed the twelve caspases found in humans. Only two of these are activated during cell death, however. The rest are apparently expressed in healthy cells, or sometimes in stressed ones, as found by Koonin. "Although it looks as though some metacaspases really are involved in cell death, they certainly seem to have biological functions that go beyond apoptosis," says Bidle.

The clearest-cut evidence for metacaspases in cell death was published this January by Patrick Gallois from the University of Manchester, UK, and his co-workers, who showed through careful genetic engineering of *Arabidopsis* that at least one metacaspase does directly mediate cell death⁵. In light of these findings, as well as some older reports from yeast and phytoplankton, Gallois thinks that metacaspases are probably "part of an ancestral pathway that activates programmed cell death in response to a damaging level of oxidative stress".


Stress relief

The tie-in with oxidative stress — a state in which there is more reactive oxygen around than a cell can tolerate — emphasizes another strong link with phytoplankton, says Assaf Vardi, a molecular biologist who joined Falkowski and Bidle at Rutgers in 2007. Ultra-violet radiation, carbon dioxide limitation, iron deficiency and viral infection can all trigger cell death in eukaryotic algae, he says. And what these things have in common are reactive oxygen species — superoxides, hydroxyls and the like. Long perceived merely as pathological by-products, these reactive oxygen species are now known to be used by cells as signals.

Vardi has found that in phytoplankton, signals from reactive oxygen species are amplified by nitric oxide. This gas does a great deal of signalling work in plants and animals, and has a key role in inflammation, immunity and cell death. Vardi is the first to show that the gas is actively generated by phytoplankton, and he has even filmed it using fluorescence microscopy.

Vardi's latest work shows that *Phaeodactylum tricornutum* — a diatom, like *T. pseudonana* — calls on an enzyme found only in chloroplasts to generate nitric oxide. Cells that overproduce that enzyme grow slowly and are highly sensitive to stress, activating as many as six metacaspases during the cell-death programme. The steps

"The plankton just dissolved overnight." — Paul Falkowski



Coccolithophores can congregate in dramatic blooms, although an artistic hand has shaped this one in the Bay of Biscay.

J. SCHWALTZ / MODIS RAPID RESPONSE TEAM / NASA / GSFC (MODIFIED BY C. DARKIN)

As most plankton in a bloom are near identical genetically, from the perspective of their genes, a die-off that creates enough scorched earth to stop the viral advance can make sense.

To understand this as self-sacrifice, though, might be to oversimplify. “It is at least as much murder as suicide,” says Vardi. “It definitely blurs the boundary between altruism and selfishness.” Vardi has found that injured phytoplankton release mediators — he calls them ‘infochemicals’ — into their surroundings. In response, damaged cells overproduce reactive oxygen species, activating the death apparatus, whereas in healthier cells the signals lead to several processes designed to deal with stress: the formation of tough, long-lasting cysts, the creation of biofilms on nearby surfaces, even differentiation into separate sexes. The system is assigning genetically identical cells different fates — rather as developmental programmes do in multicellular organisms.

Death is, in a way, the simplest form of development — a binary choice. It may be that caspases and their cousins have been called on as managers of death time and again, in shaping tissues by killing cells or in weeding out sicker plankton to benefit the rest, because these are the settings in which cells with the same genes need to thin their numbers. The programmed-death function may have been inherited from the cyanobacteria along with the metacaspases used for it. But it is also possible that the cascade-friendly features of such molecules lend themselves so well to a self-destruct sequence that they get roped in whenever such a capability is called for.

Either way, the links from today’s complex world back to the origins of death in a simpler one seem oddly fortunate. The bacterial partners in the symbioses that led to eukaryotes might not have had anything as useful as metacaspases in their make-up. They might have been getting by with some simpler death system, such as an addiction module, or no death system at all. If so, eukaryotes could have started out without the cell-death mechanisms that are so crucial to their development. As Koonin puts it: “Much of the glorious ascension to the ultimate complexity of higher plants and animals is owed to a lucky choice of bacteria with complicated differentiation processes as partners in the origin of the eukaryotic cell.” The complex seeds of death made life what it is today. ■

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1. Berman-Frank, I., Bidle, K. D., Haramaty, L. & Falkowski, P. G. *Limnol. Oceanogr.* **49**, 997–1005 (2004).
2. Uren, A. G. *et al. Mol. Cell* **6**, 961–967 (2000).
3. Koonin, E. V. & Aravind, L. *Cell Death Differ.* **9**, 394–404 (2002).
4. Bidle, K. D. & Bender, S. J. *Eukaryot. Cell* **7**, 223–236 (2008).

apoptosis. Nitric oxide blocks the electron-transport chains needed for photosynthesis and respiration; that generates reactive oxygen species, and they in turn trigger the death cascade.

This seems to show that Falkowski’s hunch was correct. Stressed phytoplankton blooms generate nitric oxide, and reactive oxygen species then activate the machinery of death. This machinery includes (but is probably not limited to) metacaspases that are functionally related to the caspases found in apoptosis in animals. But why does so much of the cell-death apparatus associated with complex animals — in which cellular self-sacrifice can make obvious sense — operate in single-celled phytoplankton? What can a plankton possibly have to gain by killing itself?

Falkowski suspects that the ultimate brokers of death are viruses. Sea water contains viruses in shocking abundance — hundreds of millions of viruses per millilitre of sea water — and phytoplankton are the targets of many if not most of them. Falkowski thinks that the death apparatus is part of an ancient tug of war between viruses and their prey.

tion modules’ found in simple bacteria such as *Escherichia coli*. In that case, viral genes in the cell encode a long-lived toxin as well as its short-lived antidote; cells that stop expressing the viral genes run out of antidote and die.

Power struggles

Cell-death systems that use metacaspases might have evolved in plankton through similar processes, Falkowski says, with viruses turning metacaspases into dangerous weapons of subjugation. Host cells then evolve new systems to wrest the viruses’ hands away from the trigger. The system might be seen as a coevolved product of the virus and the host, with each trying to control it. Last year, Bidle and Falkowski showed that viral replication can be blocked by inhibiting the death apparatus of the coccolithophore *E. huxleyi*, implying that the virus usually uses the system to kill the cell when death is to its advantage.

The odd thing is that sometimes the cells may want to turn the death programme on when the viruses want to keep it turned off. If cells can kill themselves more quickly than suits their