INSIGHTS

PERSPECTIVES

BIOCHEMISTRY

The making of a plankton toxin

A combined genetic and chemical study reveals the biosynthetic steps involved in making the toxin domoic acid

By **Georg Pohnert, Remington X. Poulin, Tim U. H. Baumeister**

Phytoplankton are unicellular photosynthetic organisms that occur in vast abundance in the world's oceans. This diverse group of algae is responsible for nearly half of the total global CO_2 fixation and forms the base of the marine food web. However, some phytoplankton taxa produce toxins that can poison marine wildlife and humans. Mass occurrences of such toxic unicellular algae in the plankton occur periodically and are known as harmful algal blooms (see the first photo). For example, the toxic diatom *Pseudo-nitzschia* (see the second photo) regularly emerges in the Pacific. In 2015, this microalga formed the largest harmful algal bloom ever recorded , which stretched along nearly the entire coast from Alaska to Mexico and caused massive ecologic and economic damage (*1*). On page 1356 of this issue, Brunson *et al.* (*2*) describe the biosynthesis of the toxin domoic acid that is produced by this diatom.

Domoic acid is the causative agent responsible for amnesic shellfish poisoning in humans after eating contaminated seafood. Interest in this toxin increased after 1987, when consumption of blue mussels from Prince Edward Island led to an incident of severe poisoning and human fatalities (*3*). Single cells of the algae produce only picogram or smaller amounts of domoic acid, but the compound accumulates as it is transferred through the food web. Mussels feeding on the diatoms enrich the toxin without being affected. Fish are also vectors that are not poisoned themselves but transfer the toxin to higher trophic levels.

In humans, domoic acid acts as an analog of the neurotransmitter glutamate in the central nervous system, leading to symptoms that range from nausea and diarrhea, to confusion and other neurological effects, before coma and death (*4*). As a safety measure,

Institute for Inorganic and Analytical Chemistry, Friedrich Schiller University Jena, Jena, Germany
Email: georg.pohnert@uni-jena.de

PHOTO: JAMES R. D. SCOTT/GETTY IMAGES

A toxic algal bloom clouds open ocean waters.

PHOTO: DIANA SARNO AT STAZIONE ZOOLOGICA NAPLES

many countries have installed surveillance programs to monitor *Pseudo-nitzschia* in coastal waters and the domoic acid concentration in seafood; fisheries are shut down and beaches closed at times of contamination. Incidents of domoic acid poisoning of sea lions with more than 400 dead animals further boosted interest in the toxin (*5*). Seabirds are also affected, and historic samples from the 1960s suggest that the inspiration for Alfred Hitchcock's famous movie *The Birds* came from crazed animals affected by domoic acid poisoning (*6*).

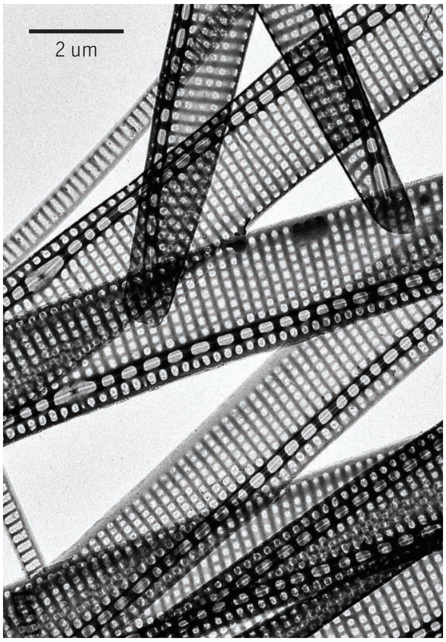
The structures of many natural toxins, including that of domoic acid, have been known for decades, but their biosynthesis is not always understood. Knowledge of how nature assembles a complex toxin can facilitate follow-up research. For example, identification of the genes responsible for biosynthesis allows the heterologous expression and modification of a toxin and drug candidate for production in large quantities, or the monitoring of toxin producers in the environment (*7*). In recent years, we have seen a number of biosyntheses that have been unraveled, including the biosynthetic pathway of the anticancer drug vinblastine and the identification of all enzymes involved in morphine biosynthesis, more than a century after its isolation in pure form in 1895 (*8, 9*). These breakthroughs have been made possible by the combination of advanced genetic methods, natural product chemistry, and physiological studies.

Brunson *et al.* use such a combined approach to uncover central steps in the biosynthesis of domoic acid in the harmful alga *Pseudo-nitzschia multiseries*. The endeavor was based on earlier observations that toxicity of the diatom increases under phosphate limitation and elevated CO_2 concentration. A comparison revealed that the transcription of ~500 genes increases under phosphate starvation. Transcription of only 43 of these genes also increased under high-CO_2 conditions; the authors used these transcripts to look for domoic acid biosynthetic genes.

The oxidase with the most pronounced increase in transcription was located in a genomic region where additional genes showed up-regulated expression. This clustering facilitated the breakthrough, because it turned out that all these genes were involved in domoic acid biosynthesis. The genes could be expressed in bacteria and yeast; application of tailored synthetic substrates, together with elaborate chemical analysis, led to the assignment of the biosynthesis of isodomoic acid. The authors have thereby uncovered the assembly line for producing an isomer of domoic acid, and in theory, this isomer can be transformed in one enzymatic step to the toxin, but the enzyme responsible is not yet known. Isodomoic acid is formed from two common primary metabolites, the amino acid glutamic acid and a terpenoid precursor geranyl pyrophosphate, in a few steps. The two precursors are first linked via *N*-prenylation and then further transformed by two oxygenases.

Knowledge of the biosynthetic pathway offers the opportunity to establish screening techniques for genetic sequences that indicate toxin production in plankton samples. These techniques will enable rapid bloom monitoring and environmental consulting. More importantly, the biosynthetic pathway could pave the way to answering scientific questions about this toxin. For example, the sequence opens the opportunity to address how metabolites with structural similarity to domoic acid are synthesized in red algae. Initial screening in these red algae has already revealed oxidases similar those required for domoic acid biosynthesis (*2*). Also, the observed gene clustering, which is not common in diatoms but widespread in fungi and bacteria, will stimulate evolutionary discussion.

Answering the more fundamental question of why the diatoms produce the toxin is now also within reach. Given that there is no direct ecological connection between mammals or birds and the unicellular algae, toxicity to the top consumers might be considered as collateral damage caused by a compound that has different importance for the unicellular producer. Many studies have been undertaken to evaluate the role of domoic acid in inhibiting competing



Electron microscopy reveals the silicified cell wall of a *Pseudo-nitzschia* diatom from the Gulf of Naples.

phytoplankton (*3*), defending against herbivorous predators (*10*), or facilitating iron uptake (*11*). However, findings have been contradictory and do not provide a conclusive picture. Knowledge of the biosynthetic pathway can be used to elucidate domoic acid's function for the diatom by studying the induction of toxin production and by studying the physiological and ecological performance of mutant strains that lack domoic acid biosynthesis genes. ■

REFERENCES

1. R. M. McCabe *et al.*, *Geophys. Res. Lett.* **43**, 10366 (2016).
2. J. K. Brunson *et al.*, *Science* **361**, 1356 (2018).
3. A. Lelong *et al.*, *Phycologia* **51**, 168 (2012).
4. K. A. Lefebvre, A. Robertson, *Toxicon* **56**, 218 (2010).
5. C. A. Scholin *et al.*, *Nature* **403**, 80 (2000).
6. S. Bargu *et al.*, *Nat. Geosci.* **5**, 2 (2012).
7. S. Galanie *et al.*, *Science* **349**, 1095 (2015).
8. L. Caputi *et al.*, *Science* **360**, 1235 (2018).
9. J. M. Hagel, P. J. Facchini, *Nat. Chem. Biol.* **6**, 273 (2010).
10. M. B. Olson, E. J. Lessard, *Harmful Algae* **9**, 540 (2010).
11. E. K. Prince *et al.*, *Mar. Drugs* **11**, 2398 (2013).

10.1126/science.aau9067

The making of a plankton toxin

Georg Pohnert, Remington X. Poulin and Tim U. H. Baumeister

Science **361** (6409), 1308-1309.
DOI: 10.1126/science.aau9067