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Profile of J. Woodland Hastings

Rippling in the night, wakes of ships leave sparkling trails in the sea, the product of innumerable single-celled dinoflagellates stimulated by the ocean's turbulence. This mesmerizing luminescence is present among many species of organisms, ranging from squid and jellyfish to bacteria, glow-worms, and fireflies. Bioluminescence has evolved a number of different times, and the enzymes and substrates that cause it, referred to as luciferases and luciferins, differ among the major organismal groups.

J. Woodland Hastings, elected to the National Academy of Sciences in 2003, reports in his Inaugural Article in this issue of PNAS on the structure of the luciferase gene in *Noctiluca scintillans*, one of the largest and most primitive bioluminescent dinoflagellates (1). Hastings' work shows that *Noctiluca*'s luciferase possesses domains for both catalysis and substrate binding, whereas in some previously described dinoflagellate species, these domains occur as separate proteins.

J. Woodland Hastings

A Glowing Start

Hastings, the Paul C. Mangelsdorf Professor of Natural Sciences at Harvard University (Cambridge, MA), was born in 1927 and spent his early years in Seaford, DE. At age 10, he became a choirboy at the Cathedral of St. John the Divine in New York City and attended the choir's in-house boarding school, visiting his family only during vacations. Moving to the Lenox School (Lenox, MA) in 1941 to complete his secondary education, Hastings came to love literature, mathematics, and physics, as well as baseball and ice hockey.

Upon graduation in 1944, Hastings enlisted in the Navy V-12 medical officers training program and joined the unit at Swarthmore College (Swarthmore, PA). Stimulated by research in Swarthmore's biology department, Hastings worked with Per Scholander and Knut Schmidt-Nielsen, measuring oxygen-consumption rates in different small organisms. "The program was accelerated because of the war," he says, "and I was scheduled to enter medical school already in the fall of 1945. But, although the war ended, the draft law required that I maintain a place in medical school." Hastings did not attend medical school however, instead completing his bachelor's degree in 1947 and resigning from the Navy, during a period when the law had lapsed. Certain that he did not want to study medicine, Hastings applied for graduate studies at Princeton University (Princeton, NJ). Still uncertain about a career track, he deferred matriculation and left the United States to teach biology at the Collège Cevenol in southern France in 1947.

Returning to Princeton in 1948, Hastings chose to work with E. Newton Harvey. "He was a major figure in the field of bioluminescence and cell physiology more generally, and a wonderful mentor," Hastings says. "Already then, prior to the detailed biochemical and molecular evidence, Harvey had perceived the evolutionary diversity and independent origins of different bioluminescent systems." Under Harvey, Hastings developed techniques to subject organisms to low oxygen concentrations as a way to measure the quantitative requirement for oxygen in the luminescent reaction of different species (2).

Receiving his doctorate in 1951, Hastings moved to Johns Hopkins University (Baltimore, MD), joining the laboratory of William McElroy, himself a former student of Harvey. Hastings recalls that McElroy had excited biochemists with his discovery that light emission required ATP in firefly extracts. "My research concerned the basic biochemical mechanism of this luciferase reaction, one finding being that coenzyme A stimulates light emission," Hastings says. "We had to have a lot of material, so we paid a penny per live firefly [to local residents]. I was in charge and paid the children for their catch."

With purified luciferase, "I obtained evidence that oxygen gating is the mechanism for firefly flashing," he says. "After its removal, the reintroduction of oxygen caused a flash with a peak approximately 100 times higher than the baseline level prior to oxygen removal, but not requiring the removal of oxygen for its decay," he explains. Hastings interpreted the flash as the result of the reaction of oxygen with a luciferase-bound intermediate accumulated in the absence of oxygen (3).

In McElroy's laboratory, Hastings also started investigating luminous bacteria and discovered a flavin to be a substrate in its luciferase reaction (4). Bacterial luciferases would later become one of the major topics of Hastings' research.

Finding a Circadian Rhythm

In 1953, Hastings accepted a faculty position in the Department of Biological Sciences at Northwestern University (Evanston, IL). He began studies of dinoflagellates after attending the first international meeting on bioluminescence in 1954 at Asilomar, in Pacific Grove, CA. There Hastings learned that Beatrice Sweeney, at the Scripps Institution of Oceanography (La Jolla, CA), had cultured a bioluminescent marine dinoflagellate, *Gonyaulax polyedra*, for the first time. Interested in elucidating the biochemistry of its bioluminescence, Hastings began an extended collaboration with Sweeney. He and Sweeney demonstrated that *Gonyaulax* had a circadian rhythm of bioluminescence (5, 6) and in ensuing years made several fundamental contributions concerning circadian rhythms.

In 1957, Hastings joined the Biochemistry Division of the Chemistry Department at the University of Illinois at Urbana–Champaign (Urbana, IL), where he studied both bacterial and dinoflagellate systems. In 1961, he became director of the summer physiology course at the Marine Biological Laboratory in Woods Hole, MA, where he had spent two summers as a graduate student. For Hastings, this experience launched an enduring association with the laboratory and its scientific community. In 1966, Hastings joined the faculty of Harvard University as Professor of Biology. Continuing work at Woods Hole, he carried out studies with coelenterate luminescence systems with graduate student James Morin. In these systems, some species emit green light *in vivo* but blue light in the isolated enzyme system.

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 696.

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In *Obelia sp.*, they found that a green fluorescent protein, previously discovered in *Aequorea sp.* (7), and which they dubbed GFP, served as a secondary emitter by virtue of energy transfer from the luciferase-bound excited state (8).

Work on the dinoflagellate *Gonyaulax* continued apace at Harvard. Graduate student Neil Krieger found luciferase fractions with different pH dependencies, foreshadowing the discovery of the regulation of the reaction by pH (9). Graduate student Margaret Fogel purified cellular organelles called scintillons and showed that when subjected to a pH jump, they emitted a living cell-like flash (10). After readjustment to pH 8.0 and incubation with fresh luciferin, the scintillons emitted a second flash. Hastings postulated that an action potential, leading to the entry of protons via voltage-activated membrane channels, triggered the pH jump in the scintillons, a mechanism now accepted.

The fact that luciferase activity increased every night and disappeared the next day remained enigmatic (11). To Hastings, it seemed possible that the cycle stemmed from activation and deactivation, for example by protein phosphorylation. Less likely, he thought, was that synthesis and destruction of the protein occurred each cycle. But the latter turned out to be the case, as shown by graduate student Jay Dunlap (12) and later confirmed by postdoctoral fellow Carl Johnson (13). The actual identity and cellular location of scintillons had escaped definition over these years. M. T. Nicolas solved the problem with immunolocalization studies, showing the scintillons to be small vesicles (0.4 μm) containing both luciferase and the luciferin-binding protein (14).

Experiments with inhibitors revealed that two classes of inhibitors affected the circadian clock mechanism, but in different ways. As shown by Dunlap and graduate student Walter Taylor, short pulses of inhibitors of protein synthesis resulted in phase shifts of the circadian rhythm, either delays or advances, depending on the time at which the pulse was administered (15). Protein phosphorylation inhibitors, however, affect the period of the rhythm (16). The importance of phosphorylation was also indicated by Till Roenneberg's discovery that a substance in extracts of the mammalian suprachiasmatic nucleus shown by Krieger to cause a striking period-shortening effect on the circadian rhythm in *Gonyaulax* is the phosphagen creatine (17).

Autoinducer Reveals Quorum Sensing

In 1970, Hastings' research provided some of the first evidence for bacterial communication and the concept of quorum sensing. He had long noted that, in newly inoculated cultures, the number of cells doubled every 30 minutes, but luminescence did not begin to increase until 2 or more hours later, when it doubled every 5 minutes. Hastings and his postdoctoral student Kenneth Nealson found an explanation for this lag (18). "The bacteria were producing and releasing into the medium a substance that turned on transcription of specific genes that had been repressed," says Hastings. "This occurred only when the concentration of this substance, which we called autoinducer. . . reached a critical level. But the result, even its reality, was widely disbelieved or ignored, and sometimes derided."

Hastings, speaking at a lecture

"We had to have a lot of [bioluminescent] material, so we paid a penny per live firefly to [local residents]."

Hastings' work with bacterial systems continued throughout the 1980s. Acceptance of quorum sensing came in the early 1990s, after the autoinducer gene was sequenced and found to occur widely in gram-negative species, where the delayed production of a toxin, for example, serves to enhance the pathogenicity of the bacterium (19, 20). As Hastings notes, "If bacteria can restrain their toxin production until their population is high, they can produce it in massive amounts in a short period of time and overwhelm the defenses of the host." According to Hastings, Nealson jokes that "[his] biggest regret is not having saved some of the referee reports from our first papers. People just thought that it was, at best, a phenomenon involving only luminous bacteria." It did explain the fact that, whereas individual luminous bacteria in the ocean do not emit light, those cultured as symbionts in light organs of fish and squid (21), where they are densely packed, do bioluminesce.

Three-Ring Circus

In 1997, Hastings and postdoctoral fellow Liming Li found that the *Gonyaulax* luciferase gene contained three homologous and contiguous repeated sequences (22). Expressed in *E. coli*, each segment coded for a peptide catalyzing the same bioluminescent reaction. "A three-ring circus with the same act in all three," Hastings quips, "and what is the reason? Maybe because this system is confined in a small organelle where protein osmotic pressure is important. So more active sites without more molecules," he explains. Postdoctoral fellow Liyun Liu examined luciferases from seven different dinoflagellate species and found them all to be like *Gonyaulax*: an N-terminal region of unknown function followed by three homologous luciferase domains (23).

The regions between tandem copies were strikingly different, with no identifiable canonical promoter sequences. Individual domains are more similar between species than between domains within species (24). The strong conservation of the amino acid sequence in the central regions of the domains indicated that these areas were active sites. Based on the crystal structure of the third domain, Hastings, with collaborator Wayne Schultz of the Hauptman–Woodward Medical Research Institute (Buffalo, NY), confirmed the location of the active site and also suggested a molecular mechanism for the regulation of activity by pH (25).

Investigating *Noctiluca*

In recent years, Hastings has studied another dinoflagellate species, the heterotroph *N. scintillans*. For some time, the luciferase gene of *Noctiluca* evaded cloning, but in his PNAS Inaugural Article with Liu, Hastings reports success in cloning the gene and the discovery that its structure, while having elements similar to other species, differs in intriguing ways (1). Alerted to a "red tide" of *Noctiluca* in the Gulf of Mexico, they immediately went to harvest them. "Wow, did we get organisms," exclaims Hastings. "In a very few minutes, the nets were teeming with *Noctiluca* and almost nothing else. We brought the frozen cells back to Harvard, but the cloning was not straightforward."

In the article, they report that *Noctiluca*'s luciferase has only a single catalytic domain, lacking residues responsible for pH control. Interestingly, a second do-

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main of the gene codes for a luciferin-binding protein-like sequence, which in *Gonyaulax* occurs as a separate gene. Thus a single protein in *Noctiluca* appears to possess both catalytic and substrate binding properties, found in separate proteins in other species (1).

Dedication to a Colleague

Hastings says that his career has been great fun, both in research and teaching, and points to a lecture he gave a few years ago at the meeting of the Society for Research on Biological Rhythms with the title "Fifty Years of Fun" (26). Praising his outstanding and dedicated students and postdoctoral fellows, only a few of whom are mentioned here, he gives them credit for much of the success of his research career. "Working on topics such as bioluminescence or circadian rhythms could only be motivated by a true interest in basic knowledge. It's not leading directly to a solution for cancer," he says.

Hastings' greatest acknowledgment goes to his long-time associate Thérèse Wilson, a member of his laboratory since their first joint publication on singlet excited molecular oxygen in 1970 (27). "She has contributed immeasurably to virtually every project and publication over these many years, while also pursuing her own photochemical research," Hastings says. "Her concern for the intellectual and social well-being of each and every lab member has greatly enhanced the experience of each, and I stand in great admiration and profound appreciation for her keen and inquisitive intellect along with her warm and engaging persona."

Tinsley H. Davis,
Freelance Science Writer

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