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# How Bad Luck & Bad Networking Cost Douglas Prasher a Nobel Prize

The discoverer of a gene for a glowing protein was driving a van for a car dealership in Huntsville, Alabama, when he learned that former colleagues had won science's greatest honor.

By Yudhijit Bhattacharjee | Monday, July 18, 2011

In December 2008 Douglas Prasher took a week off from his job driving a courtesy van at the Penney Toyota car dealership in Huntsville, Alabama, to attend the Nobel Prize ceremonies in Stockholm. It was the first vacation he and his wife, Gina, had taken in years. On the day of the awards, he donned a rented copy of the penguin suit that all male Nobel attendees are required to wear, along with a pair of leather shoes that a Huntsville store had let him borrow.

At the Nobel banquet, sitting beneath glittering chandeliers suspended from a seven-story ceiling, Prasher got his first sip of a dessert wine that he had dreamed of tasting for 30 years. When the waitress was done pouring it into his glass, he asked if she could leave the bottle at the table. She couldn't, she told him, because the staff planned to finish it later. His buddies back at Penney Toyota were going to love that story, he thought.

Prasher's trip would have been impossible without the sponsorship of biologist Martin Chalfie and chemist and biologist Roger Tsien, who not only invited the Prashers but paid for their airfare and hotel. Chalfie and Tsien, along with Osama Shimomura, an organic chemist and marine biologist, had won the 2008 Nobel Prize in Chemistry. The three researchers were sharing the $1.4 million award for the development of green fluorescent protein (GFP), a molecule that makes certain jellyfish glow. Starting in the mid-1990s, scientists began using GFP as a tracer for studying biochemical processes. The results were spectacular: The luminous protein made it possible to glimpse the inner workings of cells, tissues, and organs in unprecedented detail.

Had life turned out slightly differently, Prasher could have been attending the ceremony not as a

guest but as a laureate. More than two decades earlier, it was Prasher who cloned the gene for GFP while working as a molecular biologist at Woods Hole Oceanographic Institution in Massachusetts. The cloning was the first step in using GFP as a tracer chemical in organisms other than jellyfish. Prasher proposed an experiment to see if the GFP gene could make bacteria glow, but he was not able to pull it off. In 1992, when he was about to leave Woods Hole for another science job, he gave the gene to his colleagues Chalfie and Tsien. They went on to perform the experiments that made GFP and its variants into a powerful research tool, the foundation of a multimillion-dollar industry.

Prasher had the vision before anybody else did. But he failed to make it a reality.

If GFP's progression from an obscure protein into a biological laser pointer is a quintessential scientific success story, Prasher's journey from Woods Hole to Penney Toyota is a tale of individual and institutional failure. His vanishing act provides a glimpse into what it takes to flourish in modern-day science, where mentorship, networking, and the ability to secure funding can be as important as talent and intelligence.

And then there is the role of luck. In life as in science, small underlying variables can translate into wildly divergent outcomes. One misplaced base pair in a DNA sequence can define the gap between health and disease. The paths leading to career success or failure, too, can lie a hair's breadth apart.

At 58, Douglas Prasher sports a beard liberally flecked with gray. He's six feet tall with a paunch that invites a fair bit of ribbing from his teenage son. When I visited him at Penney Toyota on a hot and humid Friday afternoon, rows of new cars glinted under the sun, festooned with balloons bobbing in the breeze. Prasher greeted me outside the dealership's grubby-looking service center, dressed in a blue golf shirt and khaki pants—the company uniform. The courtesy van was parked across from the entrance. Squinting through sunglasses and adjusting his cap, he led me through a corridor to a body shop in the back where he introduced me to some of his colleagues. "They are all self-described rednecks," he said with a laugh.

Donny, a middle-aged man with a goatee and a golden locket, was bent over the headlight of a car with an open hood. He stood up and thrust his blackened paw out at me with a grin. "We've been teaching Douglas about the real world," he said. Jim, another body shop worker, listed some of the things they had educated Prasher about. They all happened to be local culinary delights: "mountain oysters" (hogs' testicles), fried moon pies, Goo Goo Clusters. I asked Jim if Prasher had taught them anything in return—say, about DNA. "DN *who*?" Jim asked, smiling. The comment evoked a chortle from Prasher, whose typical manner combines irony and earthiness.

Prasher was born into a working-class family in Akron, Ohio, where his father and maternal grandfather worked at the Goodyear tire factory. He too worked at the factory for a summer during college; the experience was enough for him to realize that he was not cut out for a blue-collar job. He ended up earning a Ph.D. in biochemistry from Ohio State University, guided by nothing more than a general interest in the life sciences. "I didn't know what else to study," he

told me.

After Prasher got his doctorate in 1979, he went to work as a postdoc at the University of Georgia in Athens, learning how to clone genes and get them to switch on inside bacteria. It was at the university that he met his future wife, Gina Eckenrode, a Ph.D. student in biochemistry who was drawn to his kindness and wry sense of humor, which was less cynical back then. One day when he was in the lab, she sent him a "gorillagram"—a love letter delivered by a person in a gorilla suit. Prasher still feels embarrassed when he recalls the moment. While at Georgia, he also met Milton Cormier, a biochemistry professor who was studying bioluminescence, the ability of certain organisms to produce and emit light. Through Cormier, Prasher learned about a species of jellyfish living in the cold waters of the North Pacific, *Aequorea victoria*, which emitted a green glow and was one of the most intensely bioluminescent creatures on the planet.

The fundamental chemistry behind the creature's glow had been worked out by Shimomura and others in the 1960s. The jellyfish is shaped like an umbrella, and its light comes from a ring of tiny, stemlike extensions along the umbrella's circumference. Studying liquid squeezed out from these luminescent organs, Shimomura identified two proteins—called aequorin and GFP—that worked together to emit light. Aequorin gives out blue light when it binds with calcium in seawater; this light is absorbed by GFP, which then emits an intense green glow.

Cormier had a grant from pharmaceutical giant Hoffmann–La Roche to clone the gene for aequorin. The company wanted to use it as a diagnostic marker for disease: If synthetic versions of antibodies could be tagged with aequorin in the lab, then whenever they matched up with an antigen (or surface protein) of a specific pathogen in blood or tissue, the sample would glow. The only way to make aequorin on a commercial scale, Cormier believed, was to cultivate it inside bacteria that had been genetically engineered to contain the aequorin gene. But first he needed copies of the gene; it had to be cloned. To do the cloning, he hired Prasher, who quickly became fascinated by bioluminescence.

Soon Prasher was traveling to the town of Friday Harbor, on an island in Puget Sound, to catch jellyfish by the thousands as they floated past the docks. After catching the jellyfish with pool-skimming nets, he and a group of other scientists would pin them down with a fork and spin them across a razor's edge to slice off the light-emitting photo-organs, which would fall into a bucket in a translucent linguini heap. It was exhausting work that Prasher dived into with esprit de corps. Once the team members harvested the photo-organs, they were distributed like tomatoes from a community garden.

Prasher would freeze some of this tissue right away to take back to his lab to extract DNA. Other scientists would process the same tissue further to obtain the light-emitting proteins. They would add a liter of the tissue to two liters of seawater and shake the mixture 75 times—no more, no less—to make "the individual light-producing cells pop out of the tissue," according to Bill Ward, a bioluminescence researcher at Rutgers University in New Jersey who was a post-doc in Cormier's laboratory. The cells were filtered through mosquito netting and put through another series of steps to derive aequorin and GFP.

Over the course of six months, Prasher built libraries of jellyfish genes from the tissue he collected. Every tissue in an organism contains a variety of messenger RNA molecules, single-stranded nucleotide sequences bearing instructions for the making of specific proteins. Since messenger RNA is nothing but the imprint of the gene whose message it is carrying, it is possible to use it to chemically generate the DNA sequence or gene it corresponds to. From the sizable collection of genes generated this way, Prasher hoped to identify the specific ones responsible for aequorin and GFP.

To search through his gene library, Prasher relied on work done by Ward and others, who had already partially determined the sequence of amino acids (units of a protein) of which the bioluminescent molecules were made. Working backward, Prasher created synthetic DNA molecules that were approximate blueprints of the actual genes. He tagged each artificial gene with a radioactive compound and then added it to a mixture of *E. coli* containing most of the genes extracted from the jellyfish.

As he had hoped, his synthetic genes and the real ones were similar enough for the DNA base pairs to stick together. Both the aequorin and GFP genes were now identified, but Prasher felt that just one—the gene for GFP—was biomedical gold. Most light-emitting proteins found in nature do not work alone. Instead they rely on a chromophore, a light-producing chemical unit that is analogous to the filament of a bulb. Through a complicated biochemical process, the chromophore is added on to the protein, generating light. Aequorin was like that, able to light up only with the help of its chromophore, a property scientists today call bioluminescence. GFP, however, could stand alone. Scientists called it fluorescent rather than bioluminescent because a chromophore was never needed to produce the light.

Prasher immediately grasped the importance of his discovery. As a single-unit light source, GFP could serve as a perfect molecular tag for tracking genes and proteins in an organism. If a biomolecule of interest were tagged with the GFP gene, Prasher thought, a fluorescent signal

would show when and where in the organism that gene or the protein it created was being put to use. “I knew GFP could be incredibly useful because it would be much easier to use in living systems than what was available,” he said.

All the signs looked promising for Prasher in 1987 when he got a tenure-track job at Woods Hole. He and Gina bought a house that was an eight-minute drive from the beach in the nearby town of Falmouth. The couple had a young daughter, Emma, and, for the most part, Gina stayed home to raise her. Money was always tight, but the family got by. Prasher, who likes gardening, planted vegetables in the backyard. “We’d collect seaweed from the beach to put on the asparagus bed,” Gina said. “We grew tomatoes to make spaghetti sauce.”

Prasher soon received a $200,000 grant from the American Cancer Society to clone his synthetic gene. But he hit a brick wall at the National Institutes of Health and elsewhere as he tried to fund research proving the cloned gene worked in other organisms. “It was a high-risk idea,” he told me. Many scientists in the field, including Ward and Shimomura, still doubted that only one gene was involved or that GFP could be expressed in organisms other than jellyfish.

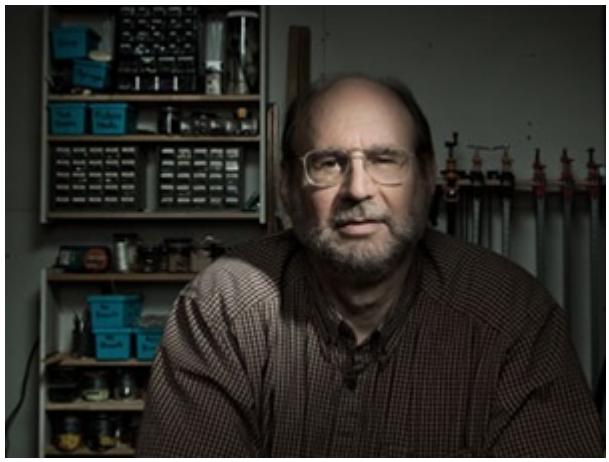
As Prasher describes it, his time at Woods Hole was a series of missed connections, psychological roadblocks, and bad breaks. He was one of only a handful of molecular biologists in a department populated by marine biologists and ecologists. “Very few people cared about what I was doing,” Prasher says. One day in 1989, he got a call from biologist Martin Chalfie at Columbia, who had heard about Prasher’s attempts to clone the GFP gene. To Chalfie, the fluorescent molecule was a potential tool to help him investigate the sense of touch in roundworms and to explore more broadly how organisms react to stimuli. Like Prasher, he also realized that GFP tags could provide a way to track the production of genes and proteins writ large. Prasher promised that he would get in touch with Chalfie once he had cloned the synthetic gene.

More than a year later, when Prasher finished cloning the gene, he called Chalfie’s lab only to learn that the researcher was on sabbatical at the University of Utah. Prasher says he left voice mail for Chalfie in Utah but never heard back; Chalfie does not recall getting any messages from him. Regardless, it wasn’t until September 1992 that Chalfie, now back at his Columbia lab, pushed forward with his GFP research. He lamented to a graduate student that he had never heard from Prasher; then a search on a computer database turned up a recent paper by Prasher reporting the cloning of the synthetic GFP gene. Within minutes, Chalfie had Prasher on the phone.

Chalfie’s call could have been a scientific lifeline for Prasher, but it came too late. Doubtful that he would be granted tenure, Prasher had already made up his mind to quit Woods Hole. A seminar he had given at the department earlier that year had not gone well. He had gotten so depressed by the lack of funding and mentorship that his daughter, Emma, who was 3 at the time, remarked one day to Gina, “Papa doesn’t smile anymore.” The next day, Prasher told the tenure committee to stop the review process and gave himself a year to find another job.

“I just didn’t fit,” he said. “I was so isolated.” He was convinced that he would struggle even if he

did get tenure; he did not have the strength to do his research in solitude when so few cared about his work. “Doug doesn’t have the ‘Goddammit, you’re not going to stop me’ attitude,” Ward says. “He’s the kind of person who really needed a facilitator-type person or organization to say, ‘Look, I think you’re doing a great job.’ ”



Along with withdrawing from the tenure process, Prasher decided to quit working on GFP. “The area of bioluminescence was esoteric work; nobody was interested, and funding was very difficult,” he says. “I didn’t want to have to struggle to find funding for something that was so difficult to convince other people to support.” He decided to pass along his research to the few who seemed to understand it. A few days after Chalfie’s call, Prasher drove to the post office with a little tube containing the GFP gene, put it in a padded envelope, and mailed it to Columbia. He also sent a sample to Roger Tsien at the University of California, San Diego, who, like Chalfie, had seen Prasher’s paper and contacted him. “He asked me if he could have the gene; I immediately said yes,” Prasher told me. “I remember he sounded very surprised.”

Leaving the post office, Prasher felt a wave of sadness. “I knew this was really the end of it for me,” he said. But passing the baton seemed like the only sensible option. Chalfie and Tsien had “resources way beyond mine, by orders of magnitude. They were both at hard-money institutions, and I was struggling to get funding. I didn’t have graduate students, didn’t have postdocs.”

Chalfie had another advantage. He did not have to apply for a grant specifically to experiment with the GFP clone; he was able to do the work as part of his project on touch sensitivity in roundworms. Within a month of receiving the gene, his group was able to make *E. coli* glow green. Chalfie’s lab did not own a fluorescence microscope and needed to monitor the results; he solved the problem in part by asking microscope salesmen to bring in demo models, which his lab hastily used for real experiments. It was the kind of resourcefulness that Prasher seemed to lack. Shortly afterward, Chalfie and his colleagues succeeded in inserting the GFP gene into the roundworm, making touch receptor cells fluoresce. The experiments confirmed that the GFP gene could make an organism light up without the need for any other molecules unique to jellyfish.

Tsien’s lab, meanwhile, tinkered with the GFP gene to see if it could be made to produce light in any other colors. By designing mutant versions of the gene that they received from Prasher, Tsien and his colleagues were able to make variants of GFP that glowed blue, cyan, and yellow, as well as a brighter green than the original GFP.

The glowing roundworm from Chalfie’s lab made the cover of *Science* in 1994. Prasher was one of the coauthors on the paper, now considered a landmark publication that helped establish GFP as

a powerful research tool. The paper would undoubtedly have boosted his chances of tenure at Woods Hole, but by the time it appeared, he had taken a job at a research center of the U.S. Department of Agriculture down the street, working on molecular detection of exotic agricultural pests. “My frank assessment is that history would be quite different if Douglas and I hadn’t lost track of each other,” Chalfie told me recently. “If we had done the experiment a few years earlier, he might have stayed at Woods Hole.”

The *Science* paper sparked a surge of interest in GFP. Prasher received dozens of requests for the gene from around the world. Although he had quit the field, he responded to every scientist who contacted him, initially sending out copies of the gene himself and later forwarding the requests to Columbia. Prasher and Chalfie were awarded a patent for the use of GFP as a marker of gene expression; in total, it earned Prasher just a few hundred thousand dollars in royalties over 15 years.

The money helped, but it was no substitute for a stable scientific career. After three years at the USDA center in Massachusetts, Prasher was transferred to Beltsville, Maryland, forcing a move that was painful for his family. “We were in the middle of the school year,” he told me. “It was rough.” Prasher did not get along with his new boss and found himself slipping into depression. But he plodded on.

In the summer of 2004, Prasher got a new job with A.Z. Technologies, a NASA contractor in Huntsville. His assignment was developing sensors to detect microbes in a spacecraft cabin during long flights. His family moved to Alabama with him. Prasher liked the work, especially because he was finally getting to collaborate in a team environment after years without it. But the unpredictable nature of science funding struck again. A year and a half later, A.Z. Technologies learned it would be losing funding for the project because of a decision by NASA to reduce support for life sciences. Prasher was laid off in the spring of 2006, and after nearly a year of unemployment—Huntsville is not exactly a biotechnology hub—he took the driving job at Penney Toyota.

On the morning of October 8, 2008, as he was fixing breakfast, Prasher heard news of the Nobel on the kitchen radio. His first reaction was to call the local radio station to tell them to correct the newscaster’s pronunciation of “Tsien.” Then he put on his Penney uniform, went in to work, and took his regular place behind the wheel of the courtesy shuttle.

After the landmark experiments with the GFP gene in the mid-1990s, “All of a sudden it became obvious that GFP was a wonderful tool,” Chalfie says. With GFP, though, as with many scientific breakthroughs, the tremendous importance of the discovery was not clear until many years after the initial work. Over the past 15 years, the GFP gene has enabled scientists to watch a plethora of previously murky biological processes in action: how nerve cells develop in the brain, how insulin-producing beta cells form in the pancreas of an embryo, how proteins are transported within cells, and how cancer cells metastasize through the body. In a press release announcing the 2008 chemistry prize, the Nobel Foundation called GFP “a guiding star for biochemistry.”

The glow of the GFP gene may have illuminated biology, but Prasher has remained in the shadows. Today he and Gina live on a quiet block in northwest Huntsville, about three miles from the Toyota dealership. In the backyard is a small vegetable patch; the day I arrived, Prasher excused himself in mid-conversation to check on "a groundhog problem" that had been plaguing his tomatoes. Walking into the house, I was struck by the handsomely furnished interior, including two ovens and two dishwashers; it didn't look like it was owned by a courtesy van driver making about $300 a week. I learned that the Prashers had begun building the house while Douglas was still working at A.Z. Technologies. They ended up using much of their savings to finish construction and were now using it to pay their monthly bills.

When he heard the announcement of the 2008 chemistry Nobel, Prasher felt angry and disappointed, not because he had missed out on the prize but because he was "out of science and out of a job" that paid enough. In media interviews days after the prize was announced, he jokingly said that he was accepting donations. A few checks did come in from sympathizers, including $1,000 from a lady whom Prasher had driven in his van. Prasher put a sticker on the back of his own car that said "Scientist needs work" and listed his phone number. He removed it only when Karl, his teenage son, made a fuss.

On the second morning of my visit, during which I stayed at Prasher's house, I woke to find my host padding about the kitchen in sweatpants. He was making a semolina porridge that he and Gina had first tried at the breakfast bar of the Grand Hotel in Stockholm, during his Nobel trip. A book titled *Winning the Games Scientists Play* lay on the kitchen counter next to a framed clip from *The Huntsville Times* headlined "Local Scientist Misses Nobel."

The attention brought by the Nobel Prize left Prasher in a strange emotional place: pleased that his work had not been in vain, yet sullen about the way things had turned out for him. Despite his flippant request for donations, he seemed inclined to rebuff any pity with scorn. After the Nobel ceremonies, Tsien asked Prasher if he would be interested in joining his lab at UCSD. Prasher declined. It felt like a handout, he told me; people would say, "Douglas can't go anyplace else, so Roger's picking him up." Another reason, he said, was that he wanted to distance himself from GFP. "That's in my past. That's over," he said, as if the protein were an addiction he was intent on ridding from his life.

An aura of "what if" hangs over Prasher's career. "I don't know why Douglas didn't get help from his former mentor, Milton Cormier," Tsien says. Cormier, now retired, told me he "had no idea that Doug had given the GFP gene to Roger or Martin and that he had left Woods Hole until it was all over. This was too bad; if Doug had confided in me, I could have gotten him a position in the pharmaceutical industry."

What about reaching out to scientists at Woods Hole, particularly Shimomura, whose work overlapped his own? "It never occurred to me," Prasher says, betraying what seemed like ignorance of the need to network in any field, especially science. "He kept to himself and I kept to myself." It seemed like a strange stance for somebody who didn't want to work in isolation. I also wondered if Prasher could have sought help from some of the scientists who contacted him for the

GFP clone. “He doesn’t like asking for favors,” Gina told me.

One of Prasher’s interests is Civil War reenactments, and from his perch in 2011, he imagines a reenactment of his early career: If only he had networked with other scientists and institutions, especially when he felt isolated, he says today, he might have stayed in the field of bioluminescence or at least in science.

Many of the challenges Prasher faced were hardly unique to him. Scientific opportunities often appear only at specific times and places, potentially a serious impediment for a parent who doesn’t want to relocate the family. Do your work in the wrong place, or publish it in the wrong journal, and it may vanish without a trace. And once someone drops out of science, it is hard to get back in. After joining the Toyota dealership, Prasher applied for a couple of science-related jobs in Huntsville, but nothing worked out. On one occasion he had an encouraging meeting with the hiring manager at a local company working on microfluidics; when the interviewer learned that Prasher drove a courtesy van, his interest cooled. There is no way to know how many other potential researchers were driven from their studies for similar reasons, or how many potential discoveries were never made because of the psychological and practical difficulties of the scientific lifestyle.

Finally in June 2010, several weeks after my visit, Prasher’s luck changed. He e-mailed me to say he’d been offered a science job at Streamline Automation, a local research and development company. Staffed by about 20 people, the company does work for NASA, the National Science Foundation, and the U.S. Department of Defense. Prasher’s first task when he started in late June was to help develop technology to sense toxic industrial gases.

He began cautiously. “There was none of the tremendous relief you might expect,” he says. “I had been so discouraged over the years that my attitude was, this may work out and it may not.” Gradually he settled into the job. At home he occasionally took science reading to bed, something he hadn’t done in years. “A lot of the hangdog is gone,” Gina told me. In December Prasher won a six-month, $70,000 grant from the Department of Defense to develop a field technique for categorizing tick specimens according to their mitochondrial genes, in hopes of limiting the diseases a doctor might diagnose. It brought a sense of accomplishment that had been missing from his life for a long time. In January he told me that the cloud of depression he had lived under for years was finally lifting. Science gave him a sense of purpose.

Still, there were things about Penney Toyota that he would remember fondly, he told me: the time he spent with his buddies in the body shop, their amusement when he showed up for his first day of work dressed in suspenders, the endless teasing about Prasher’s liberal views as they watched Fox News around the clock, the question one of them asked when Prasher was about to slice open a kiwi at lunch: “Are you going to eat that fuzzy potater?” These colleagues had broadened Prasher’s definition of expertise. “Even though these people are not in a glamorous position, they are incredibly knowledgeable about what they do,” he says. In our most recent conversation, Prasher told me he was busy reading up on a new technique he could apply to the detection of food allergens. “One thing I’ve always enjoyed is browsing the literature,” he says, noting that he

prefers thumbing through journals to doing keyword searches because it allows him to discover concepts he hadn't known about before. For several years, he notes wryly, "there was very little motivation to do that." Now there was, a reason to step back into a world of inquiry where the pursuit of an exciting idea is its own reward. After an untimely and seemingly permanent exit from science, Douglas Prasher has made his way back in, a personal triumph no less meaningful to him than a Nobel Prize.