

Profile of Susan S. Golden

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Susan Golden did not set out to become an expert in biological clocks, the internal timepieces that keep life on Earth adjusted to a 24-hour cycle. Instead, Golden, elected in 2010 to the National Academy of Sciences, wanted to identify the genes that underpin photosynthesis. However, her focus changed in 1986 with the discovery of biological clocks in cyanobacteria (1).

Because cyanobacteria are among Earth's earliest living organisms, the discovery made clear that biological clocks are evolutionarily ancient. Golden had been studying photosynthesis in cyanobacteria since graduate school. Her reason was simple: Cyanobacteria are single-celled, and thus they are much easier to manipulate in a laboratory than plants. With her expertise in cyanobacteria, Golden found herself well-positioned to identify the genes and proteins that make the clock tick. "Cyanobacteria are present in niches and habitats all over the planet," she says. "They're very good at adapting to their habitat and that adaptation involves doing things at the right time." Their timekeeping ability, she adds, helps cyanobacteria generate about 30% of atmospheric oxygen.

Over the years, Golden and her collaborators have revealed that the clock in cyanobacteria works like a mechanical clock, complete with oscillators, gears, and hands. In her recent research, including her Inaugural Article, Golden has shown how clock proteins interact to synchronize the internal clock with the external 24-hour cycle (2, 3). To Golden's own surprise, this recent line of inquiry has taken her back to her roots in photosynthesis. Her findings show that cyanobacteria don't use sensory photoreceptors, proteins that convey light-related information into the body in mammals, to set their clocks. Instead, cyanobacteria integrate the ability to tell time with their photosynthetic apparatus. In retrospect, says Golden, this finding makes sense: In the prokaryote's world, she says, "If photosynthesis is running, the lights are on. It's daytime. If photosynthesis is not running, it's dark."

An Unlikely Path to the Academy

The roots of Golden's career in plant biology were not formed at an early age. Instead,

Golden pursued other passions. She loved literature, she says, an interest gleaned from her mother, an avid reader. And she played the bassoon in the school band—where she made most of her friends. "That turned out to be a social bifurcation. I did not realize that the band route is the nerd route. You can't break over into the other [cool] group," Golden says. She also worked on the school newspaper as a photographer, one, she is quick to note, without any formal training. By the time she graduated high school in 1976 as salutatorian of her 600-student class, Golden harbored dreams of becoming a photojournalist for *Life* magazine or *National Geographic*.

Golden's main consideration in selecting a college, though, was not prestige or program of study but money. By this point, Golden's parents were divorced and her mother shouldered her expenses. "When I found out how much my mother made, I was shocked by how poor we were," Golden says. So when Mississippi University for Women in Columbus offered her a place, Golden welcomed the opportunity. However, after only a day of classes, Golden realized that she didn't want to be a journalist. Instead, Golden found herself loving introductory biology and soon switched her major to biology with a minor in chemistry.

With several advanced placement credits from high school and packed course loads in college, Golden graduated from college in just 2 years, unsure of her future. Fortunately, she was accepted into a PhD program at the University of Missouri-Columbia. She became one of the school's first trainees in a new cell and molecular biology training program funded by the National Institutes of Health.

For the Love of Chloroplasts

As an undergraduate student, Golden had eagerly followed a once-fervent debate about whether or not to allow recombinant DNA research to continue. Despite the potential to fundamentally alter the course of genetic research, many researchers feared that genetic engineering could inadvertently change innocuous microbes into pathogens (4). At Missouri, Golden realized that she



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wanted to perform molecular biology research. So Golden joined the laboratory of Lou Sherman, who had earlier invited her to enter UMC.

Besides providing her with academic focus, graduate school brought Golden another surprise: a husband. James Golden, another NIH trainee, soon became Golden's role model. Despite decades of running adjacent laboratories, only recently have their lines of research converged. When they moved to the University of California at San Diego in 2008, says Golden, their laboratories joined forces in researching cyanobacteria's potential as a biofuel.

In Sherman's laboratory, Golden's charge was to develop a genetic system that could be used to find the proteins that make up the photosynthetic reaction center in cyanobacteria. The approach relied on a strategy of identifying mutant photosynthesis proteins that cause cells to become resistant to herbicides like atrazine (5). Golden continued the work when she entered Robert Haselkorn's laboratory at the University of Chicago in Illinois in 1983. By then,

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 17765 in issue 44 of volume 109.

researchers had isolated the gene thought to be responsible for herbicide resistance, but they could not genetically engineer chloroplasts to confirm their theory. Golden, however, was able to mutate the suspected gene, *psbA*, and reinsert it into cyanobacteria. She then exposed the mutants to the herbicide and, sure enough, they withstood the assault. “We could show . . . that this really is the herbicide resistance gene,” Golden says (6).

The findings demonstrated that genetic manipulation is easy in cyanobacteria, making it a suitable model organism. During her postdoctoral fellowship in Chicago, when she began studying gene regulation, Golden found that cyanobacteria housed a family of three *psbA* genes (7). The three genes coded for two forms of the protein, one that mutated to confer resistance to the herbicide, and two that seemed to code for the same protein. Why, Golden wondered, did cyanobacteria house those redundant genes? Over the next several years her research group found that the genes encoding one form of the protein were expressed only under high luminance and the other expressed only under low luminance (8). Further analysis revealed that cells optimize their physiology by using the more efficient version of the protein under low stress conditions and switching to the high-stress option when conditions become unfavorable.

Turning to Clocks

When Golden accepted a faculty position at Texas A&M University in College Station in 1986, she had no intention of studying biological clocks. “I was definitely not looking at the clock. I was studying the regulation of photosynthesis genes by light,” she says. However, unwittingly, Golden discovered a sought-after method for studying the clock.

Her laboratory, Golden says, was searching for mutations that caused the light regulation of photosynthesis genes to break down. To find such mutations, she needed a way to measure gene expression in living cells. Such an approach would let her approximate how much a gene is expressed under certain light conditions and how that expression changes under other conditions. She could then identify mutants that failed to regulate properly. Golden attached the genes she was studying to a luciferase gene, similar to the gene that causes fireflies to light up. She then borrowed a night vision scope from a colleague and eyeballed her cyanobacteria. Sure enough, light emissions from the bacteria placed in the dark changed when the cells were first subjected to different intensities of light.

Golden’s work caught the attention of Carl Johnson, a circadian rhythm researcher at Vanderbilt University in Nashville, Tennessee. Johnson wanted to know whether Golden had ever studied circadian rhythms in cyanobacteria. Golden recalled that a graduate student of hers had noticed “funny oscillations” in gene expression of cyanobacteria grown for several days. “We might have actually seen some evidence of that,” Golden informed Johnson. She sent him a strain of the luciferase-tagged cyanobacteria, and Johnson, together with Takao Kondo, a researcher now at Nagoya University in Japan, observed bioluminescence from the cyanobacteria increase during the day and drop at night. The cyanobacteria’s light production continued to oscillate even outside the day-night cycle. The researchers were ecstatic. At first, Golden failed to understand the excitement.

It didn’t take long, though, for Golden to become enamored of the world of circadian biology. Johnson and Kondo explained that they had fashioned the first known bacterial system in which they could isolate and study the components of the clock. The team published their findings in 1993, noting that the discovery of the clock in a prokaryote could explain how biological clocks evolved (9).

The three laboratories, joined by Masahiro Ishiura in Japan, began working together to identify components of the clock. Kondo developed a screening device to sift through thousands of cyanobacterial colonies with mutated clocks (10). The researchers identified mutants that miscounted a day as having less than 24 hours or more than 24 hours, as well as mutants with no rhythm at all. Golden’s tools for the genetic manipulation of cyanobacteria enabled the group to fix the mutants to reset the clock to a 24-hour cycle. That approach helped the group identify three genes—*kaiA*, *kaiB*, and *kaiC*—that code for oscillator proteins that keep the clock on time (11).

Golden then looked for secondary components of the clock, or proteins that indirectly influence circadian rhythms. For instance, Golden found that knocking out a gene called *sasA* threw the cyanobacterial clock out of kilter (12). *SasA* is critical for relaying time-related information to the rest of the cell, much like the hands of a clock that help us tell time, Golden says.

Return to Photosynthesis

Before long, Golden began investigating how light-related information enters the clock. How is it, she mused, that the cell reads cues in its environment to stay calibrated

to a 24-hour cycle? Ultimately, the work led to Golden’s suite of findings detailing how the clock and photosynthesis are intertwined in cyanobacteria.

Golden knew that she could change time zones in her cyanobacteria by exposing them to constant light, followed by a pulse of darkness. However, when Golden mutated a gene called *cikA*, the dark pulse caused no change (13). “If you don’t have *CikA*, you still have a pretty good clock, but you can’t reset it,” Golden says. “The cells have permanent jet lag.”

Next, Golden analyzed the structure of *CikA*, revealing that *CikA* contained a protein domain that resembled another domain in *KaiA* (14, 15). Golden realized that *KaiA* might also play a fundamental role in setting the clock. Golden found that *CikA* and *KaiA* bind to quinones, molecules that carry electrons. In photosynthesis, quinones have few electrons (oxidized) when the lights are off but gain electrons (reduced) when the lights come on. Golden showed that the redox state of quinones influences *KaiA* activity: When oxidized, as is the case when cells go into the dark, *KaiA* dislodges from *KaiC*—the master protein of the clock—and binds quinones, thus resetting the clock. The clock behaves “just as if you hit a light switch,” Golden says (2, 3).

The finding, laid out in Golden’s Inaugural Article, expands an earlier finding showing a regulatory relationship between *KaiA* and *KaiC*. *KaiA* stimulates *KaiC* to phosphorylate, or transfer a phosphate group to a protein from ATP, the cell’s energy source (15). By controlling that process with quinone binding, *KaiA* can convey luminance-related information to *KaiC*. Additionally, *KaiC* can sense the ratio of ATP/ADP, which also changes when cells enter darkness (16). “With these two different ways of sensing photosynthesis, the [*KaiC*] oscillator can sense both when the lights go off and how long they’re off,” Golden says.

Just as cyanobacteria don’t directly sense light via photoreceptors, the human body has many organs that use clocks that don’t rely on light. For instance, the liver sets its clock not through light but through feeding. Perhaps these peripheral clocks work like the clock in cyanobacteria and use energy transfers to stay synchronized, Golden speculates.

Finding the Clock’s Hands

As Golden unravels the secrets underlying the clock, her questions have broadened. How, for instance, does the clock control the timing of cell division? And how do cells inherit a sense of time? Having established

how light-related information enters the clock, she now wants to determine how time-related information exits it.

Golden's research into the mechanisms of photosynthesis and the biological clock has earned her accolades throughout her career, including a National Science Foundation Presidential Young Investigator Award from 1989 to 1995 and a fellowship in the American Academy of Microbiology in 2000. Golden also helped start the Center for

Chronobiology at UCSD, where she now serves as director.

Besides investigating what makes the cyanobacterial clock tick, Golden has begun taking her research in a new direction. At UCSD's Center for Algae Biotechnology, Golden is helping to assess the feasibility of using cyanobacteria as a biofuel. "Cyanobacteria are already really good at converting light energy to chemical energy. That's what they do for a living," she says.

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