Profile of Sabeeha Merchant

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Organic molecules dominate the biological world, yet many of life's critical molecular reactions depend on metals for catalysis. Sabeeha Merchant, a professor of biochemistry at the University of California, Los Angeles (UCLA), studies how cells keep track of crucial trace nutrients like iron and copper. Her early work on *Chlamydomonas reinhardtii*, a single-celled alga that, like all plants, primarily uses a copper-based electron transporter during photosynthesis, revealed that the organism engages an alternate, iron-dependent molecule when copper availability is low (1). Merchant, elected to the National Academy of Sciences in 2012, says, "I always get asked, 'Why have two pathways?'" In her Inaugural Article, Merchant provides experimental support for plastocyanin as a de facto copper storage molecule. The protein is active during times of copper sufficiency but gets degraded to liberate its copper stores for other essential needs when the backup pathway is initiated in copper-deficient environments (2).

There's No Escaping Biology

Merchant, born in 1959, grew up in Mumbai, India, where students often choose an area of specialization by age 12. Her focus on science proved the result of aptitude rather than interest. "Which 12-year-old knows what he or she wants to do?" she says. "Only a few, and the rest of us took so-called aptitude tests in various subjects. I tested positive for both humanities and science and ended up in the science track because it didn't have enough girls."

An excellent student, Merchant liked chemistry, math, and physics but not biology. "I could handle the earthworm dissections but not the cockroach or frog." She started university at age 15 in India, although her family soon relocated to the United States when her younger brother graduated from high school and decided to pursue engineering. The family moved near relatives to Whitewater, WI, going from "a city of 10 million to 10,000, most of those students. We had nothing to do," she recalls. "I quickly figured out that I needed to transfer." To convince her mother, Merchant changed her major from chemistry to molecular biology. "It was a new major at the time that most small schools didn't have."

Merchant entered the University of Wisconsin, Madison, in 1978 and discovered that taking courses in a wide range of subjects provided an enjoyable change from her early schooling, with nonscience courses like logic shaping her analytical skills. "I had to take all of the biology that I avoided in high school." This time, however, she found courses that piqued her interest in the chemistry of living cells. "That's when things clicked," says Merchant, a chemist at heart. Merchant, who still did not think of science as a career, also appreciated being exposed to research. Just before graduating, in 1979, she took a job as biochemist Henry Lardy's secretary in the Enzyme Institute. During her tenure, Merchant began to perform experiments in the laboratory.

Eventually, with Lardy's insistence, Merchant applied and gained acceptance to biochemistry graduate programs across the country. She chose to stay at Wisconsin because it seemed student centered. Merchant undertook her doctoral research with Bruce Selman, who had just started studying *Chlamydomonas reinhardtii*, a ubiquitous single-celled alga, relatives of which grow even in snow or sewage. The cells' ability to grow and build a photosynthetic apparatus in the absence of light makes it an attractive system to study the components of photosynthesis because the pathway is not required for survival. Merchant purified and characterized Chloroplast Coupling Factor 1, a critical component of the photosynthetic pathway that synthesizes ATP, the cellular energy currency, from the proton gradient (3) and showed that the $\alpha_3\beta_3$ subunit stoichiometry of bacteria and mitochondria is conserved in chloroplasts as well (4).

Fresh Opportunity

For her postdoctoral fellowship, Merchant wanted to learn molecular biology and chose to work with Lawrence Bogorad at Harvard University. In 1984, she began working on light-regulated gene expression, but 4 months later, a devastating fire destroyed the laboratory. With no projects running, Merchant spent the time when she wasn't setting up the new laboratory in the library reading papers. "I always tell students that going to the library is a really important part of being a scientist," she says. "Though now it just comes to you on your computer." During these hours in the library, Merchant found a 1978 paper by biochemist Paul Wood showing that *Chlamydomonas* preferentially uses plastocyanin, a copper protein, in the photosynthetic electron transport chain when copper is available and switches to cytochrome c_6, an iron-containing protein, if copper is low (5).

Sabeeha Merchant. Image courtesy of Reed Hutchinson.

Intrigued, Merchant approached Bogorad with her interest in studying how cells detect copper levels. "Nobody was studying metals as regulators at that time," says Merchant. She eagerly undertook the project and made inroads in just a few months, generating monospecific antibodies against both proteins in rabbits; 30 years later, Merchant distributes the antibodies globally and uses them in her own work.

Merchant showed that RNA for *C. reinhardtii* plastocyanin is always present but the

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 2644.

www.pnas.org/cgi/doi/10.1073/pnas.1500798112

protein is only produced when copper is available. Pulse chase experiments revealed that plastocyanin is also translated all of the time but degraded in low copper conditions (6). She found that cytochrome c_6 RNA, however, is only present in copper-deficient cells (1). This alternate, iron-based protein only appeared when the cell called for reinforcements. Chlamydomonas had two means to the same end, a concept that would provide fruitful investigative avenues for years to come. "The concept of a backup protein did not exist at that time," she says.

Separating Contamination

Merchant joined the UCLA faculty in 1987. Merchant recalls her relief that the offer at UCLA included a water purification system, uncommon at the time, to filter out trace metals. Studying metal nutrient deficiency requires following strict protocols to ensure that no stray metal ions contaminate the experiments. "It takes me a long time to train students and post docs," she says. Iron is "easy to get rid of because it's not bioavailable," but other metals are difficult. Most water pipes are copper, hence the need for ultrapure water.

To mitigate contamination, Merchant's laboratory also acid washes all glassware with high concentrations of hydrochloric acid, dislodging metal ions from the surface with protons. They use plastic wherever possible, but they "need glass for the algal cells because they like light," something the opaque plastics cannot provide. Fortunately, *Chlamydomonas* can grow on water and a few salts, allowing Merchant to create nutrient-deficient environments easily. Chelators can sequester metals, but "physically the element is there, it's just harder for the cells to get," she explains. "I think it's not the same biology looking at a deficiency versus the presence of a chelator." Merchant's Inaugural Article thus provides a quantitative look at copper deficiency as well as a return to her roots.

Tracing Copper

In the beginning, copper-responsive signaling constituted the core of Merchant's work. After studies of a mutant lacking plastocyanin showed that the cells respond directly to the concentration of copper in the medium, Merchant continued to search for the mechanism behind copper sensing (7, 8). Eventually, she discovered that the same master regulator, CRR1, controls plastocyanin and cytochrome c_6 accumulation, albeit via different mechanisms (9). Through the 1990s, Merchant, with departmental colleague Todd Yeates, focused on structural and genetic aspects of plastocyanin and its iron-containing alternate cytochrome c_6 in parallel. In addition to solving the crystal structures of both proteins (10, 11), they teased apart the genetic pathways to study the proteins' biosynthesis and expression.

Merchant now estimates that copper represents only 25% of the laboratory's efforts, having broadened her focus to explore iron sensing and the role of iron limitation on photosynthesis (12). "Iron is much more fundable than copper work," she says. "There are 100 times more iron proteins than copper, therefore there are more diseases and nutritional issues associated with iron-deficiency." The difference is attributable to the elements' representation as life evolved on earth. "Copper is new to biology," explains Merchant. "It's younger than iron," having become a bioavailable redox agent only after the introduction of oxygen into the earth's changing atmosphere, an event that also transformed iron into its less bioavailable oxidized form (13).

Shifts in Evolution

The relative paucity of copper proteins, however, makes copper attractive for studying trace metals. Merchants notes that the work in her Inaugural Article, which follows copper allocation during shifts in copper availability, was only possible because there are so few copper-containing proteins in the cell, greatly reducing the number of labor-intensive fractionations required to separate them. Merchant designed the experiment to replicate what algal cells might experience in nature during an algal bloom, from the initial phase, with low cell density and high copper availability, to high cell density and copper scarcity. The team used cell fractionation and inductively coupled plasma mass spectrometry to obtain the elemental profile of cells and cell fractions in each nutrient profile and showed that copper from the plastocyanin fraction moves to the cytochrome oxidase fraction when cells grow in copper-deficient environments (2). The work underscores her thinking that plastocyanin functions as a copper reservoir and perhaps gives cells a competitive advantage in a copper-deficient environment.

"My research has definitely taken a biological turn. Now we do a lot of genomics," she says. She led a team that sequenced the *Chlamydomonas* genome in 2007, and her laboratory is working on sequencing extremophiles (14). Extremophiles' responses to metal availability pique Merchant's interest because many of these organisms live at extremes of the pH scale, and metal availability is exquisitely pH dependent. She expects the work will keep her occupied, especially as she explores extremophiles living in nutrient-scarce environments like the Arctic.

"I used to have hobbies," says Merchant, who will become Editor-in-Chief of *The Plant Cell* in 2015. "I think we get much busier when we are older. My so-called free time has a lot more committees."

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