Profile of Aziz Sancar

When biochemist Aziz Sancar started his doctoral studies in molecular biology at the University of Texas at Dallas (Dallas, TX), he knew he faced an uphill battle. Trained as a physician in Turkey, which at that time did not have the resources for rigorous training in research science, Sancar came to the United States believing he would not be as skilled at the laboratory bench as other students. "To compensate for this deficiency," he says, "I worked very hard and designed technically simple experiments that would go to the heart of the problem."

When one of his early, "simple" experiments repeatedly failed, Sancar began to lose confidence in some of his abilities. One day the situation worsened to the point where his benchmate told him, "Aziz, you have no talent for experimental research. I understand you were a good medical doctor; why don't you go back to practicing medicine?" Nevertheless, Sancar persisted, and his efforts have been successful, as evidenced by his 30-year research career covering DNA repair, cell cycle checkpoints, and the circadian clock. His longest-running study has involved photolyase and the mechanisms of photoreactivation. In his Inaugural Article in this issue of PNAS, Sancar captures the elusive photolyase radicals he has chased for nearly 20 years, thus providing direct observation of the photocycle for thymine dimer repair (1).

Currently Sarah Graham Kenan Professor of Biochemistry at the University of North Carolina School of Medicine (Chapel Hill, NC), Sancar has employed a strategy of hard work, perseverance, and technical simplicity in his science. His honors include the Presidential Young Investigator Award from the National Science Foundation (1984) and the highest awards from the American Society for Photobiology (1990) and the Turkish Scientific Research Council (1995). Sancar, the first Turkish-American member of the National Academy of Sciences, as well as its first University of Texas at Dallas alumnus, was elected in 2005.

Goal Keeping

Sancar was born the seventh of eight children in 1946 in the small town of Savur in southeast Turkey. "My parents were both illiterate," he says, "but they valued the importance of education and did their best to ensure that all of their children would receive some education." Sancar studied hard in school and played hard on the soccer field. During his senior year of high school, Sancar, who played goalkeeper, was invited to attend tryouts for Turkey's national under-18 soccer team. "This was a dream come true because, since the age of 7, I had wanted to play for the national team," he says. "However, upon serious consideration, I decided I wasn't tall enough to be an outstanding goalie, and instead I concentrated on my studies."

Aziz Sancar

"What we lacked with resources, we made up with enthusiasm."

Sancar excelled in many scientific disciplines in high school, and, after graduating, he narrowed his career choices to chemistry or medicine. He scored high enough on his university entrance examinations to attend the school of his choice in Turkey, and he entered Istanbul Medical School (Istanbul, Turkey) in 1963. Sancar remembers how his basic science professors conveyed the excitement of scientific discovery extremely well. "What we lacked with resources, we made up with enthusiasm," he says.

After taking a biochemistry class during his second year of the six-year program and becoming highly interested in the concepts learned, Sancar decided to become a research biochemist. When he discussed his desire to pursue a Ph.D. with his biochemistry professor, however, Sancar was advised to practice medicine, at least for a little while. In the opinion of his professor, "anyone who gets a medical degree and gets all this training should practice for a couple of years before going into the basic sciences," says Sancar. Even though he had already made up his mind, he followed the advice and spent two rewarding years as a rural physician near his hometown of Savur.

After concluding his medical practice in 1971, Sancar hoped to continue his biochemistry training in the United States, a desire somewhat paralleled by a famous foreigner. "I recall around the time I arrived, John Lennon was also trying to come to the U.S., and the INS would not allow him in because he had a conviction for marijuana use," Sancar says. "When Lennon was asked why he was so determined to come to the States, he said something like if he'd lived during the time of the Roman Empire, he would want to go to Rome because that's where the action was, and at the time the action was in the United States."

In Sancar's case, he had become interested in the phenomenon of photoreactivation, whereby DNA damage caused by UV light can be repaired by longer-wavelength blue light. This reaction is mediated by the enzyme photolyase, which was identified years earlier by Claud Rupert at Johns Hopkins University (Baltimore, MD). At the time, in 1973, Rupert was teaching at the University of Texas at Dallas, and Sancar joined his laboratory and the university's molecular biology program.

A Scientist, a Technician, a Wordsmith

Rupert was an ideal advisor for Sancar. "He understood my capabilities and limitations," says Sancar. "He encouraged me, gave me advice, and pointed me in the right direction. But, most importantly, he gave me the freedom to develop my own ideas and test them. As both a scientist and a gentleman, he has been the most influential person in my career."

When Sancar joined Rupert's group, the major question regarding photolyase was the nature of its chromophore, a question Sancar became obsessed with

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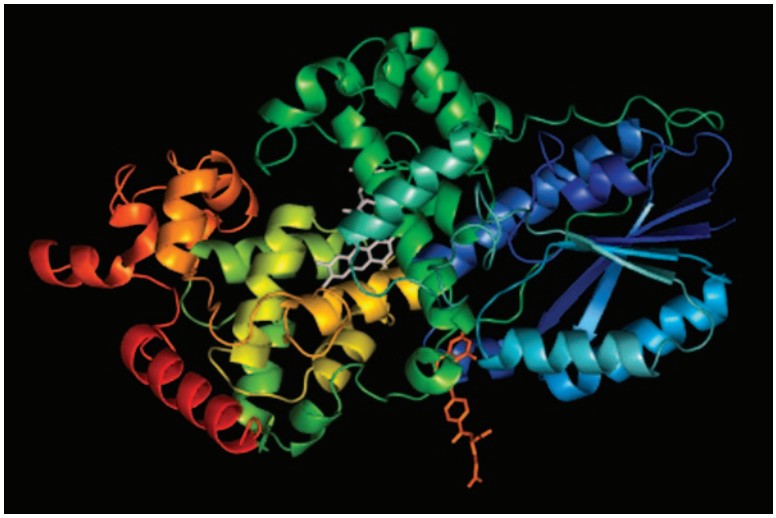
This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 16128.



E. coli photolyase crystal structure.

answering. "I told a fellow graduate student that I was willing to give my right arm to identify the chromophore, and I meant it!" says Sancar. Before taking such drastic steps, however, he tried an experimental approach to the problem. "About the time I started my research, recombinant DNA was born, and I realized I could use this technology to overproduce photolyase and identify the chromophore," he says. "All I had to do was clone the gene into a multicopy plasmid. However, to do that, I first needed an *E. coli* mutant lacking photolyase."

Sancar devised a conceptually simple method to isolate photolyase-deficient mutants, which involved damaging bacterial cells with germinated UV light and then restoring them with normal light. He notes that the method was simpler in concept than in execution, because it did not work on the first, second, or third try. Sancar persisted, and, 11 months after his first attempt, he managed to isolate a photolyase-deficient *phr*$^-$ mutant strain (2). He considers that experiment the one that truly made him a scientist. "It reinforced my conviction that I had the ability to gather disparate facts from several fields to create a hypothesis, enough technical ability to carry out the experiments, and the perseverance to continue in the face of adversity," he says.

After that breakthrough, Sancar proceeded to clone the photolyase gene in the spring of 1976 (3). "I believe it was the first gene to be cloned east of the Rockies," he says wryly. "At least that's what I tell my students to impress them." After a four-month return to Turkey to perform compulsory military service, Second Lieutenant Sancar returned to Texas to finish characterizing the cloned photolyase gene. He had hoped to purify the protein as well, but Rupert told him he had done enough to write his Ph.D. dissertation and graduate.

Although graduating was fairly simple, moving on proved difficult. Sancar had hoped to continue studying DNA repair, but all three laboratories he applied to rejected him. Sancar's fiancée, fellow graduate student Gwendolyn Boles, had secured a position in New York. "Fortunately, I learned that Dean Rupp at Yale was interested in cloning repair genes, so I contacted him," says Sancar. Although Rupp did not have a postdoctoral position available, he had a technician vacancy, and Sancar was hired, nominally, as a technician in 1977.

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Like Rupert, Rupp proved a valuable mentor who further contributed to Sancar's growth as a researcher. As in Dallas, Sancar had managed to land in the middle of the action. "Besides Rupp, Yale had other pioneers of DNA repair such as Paul Howard-Flanders, who helped discover excision repair and recombinational repair, Frank Hutchinson, and Charles Radding," says Sancar. "Yale was one of, if not *the*, center for DNA repair."

Feeding off this exciting environment, Sancar identified and cloned several *E. coli* repair genes within two years, including the *uvrA*, *uvrB*, and *uvrC* genes involved in excision repair (4–6). Armed with his newly cloned genes, Sancar purified the three Uvr proteins and reconstructed the mechanism of excision repair. To Sancar's surprise, the complex did not just nick the DNA near the damage, which was a popular working model at the time, but instead made a cut on each side to excise a chunk of DNA (7). Sancar termed the enzyme for this activity excision nuclease, or excinuclease. With Rupp's help, Sancar also invented a method for identifying plasmid-encoded proteins through bacterial cells called maxicells (8). These maxicells were critical to his success in purifying the Uvr proteins. Within days of publishing his paper on maxicells in the *Journal of Bacteriology* in 1979, Sancar received more than 100 letters requesting his new cells, and he joyfully plastered these letters all over Rupp's office. To this day, Sancar's maxicell paper remains his most cited. Besides advancing science, these two studies secured the terms "excinuclease" and "maxicell" as entries in the Oxford Dictionary of Biochemistry and Molecular Biology.

Return to Photolyase

In 1982, Sancar received an offer to join the faculty at the University of North Carolina (Chapel Hill, NC) as an Associate Professor of Biochemistry. By that time, his mentor, Rupert, had left research to become the Dean of Arts and Sciences of the University of Texas at Dallas, and his departure allowed Sancar to resume his work on photolyase. Sancar joined the University of North Carolina, and, together with Boles and other collaborators, he identified the photolyase's long sought-after chromophore—both of them, in fact (9–11). "I was expecting one, and I found two," Sancar says. "One is FADH$^-$, and the other is a pterin." Sancar developed a model for the reaction mechanism of photolyase repair (12–14) but had difficulty proving his scheme because he could not experimentally capture the proposed radical intermediates. "I worked with ultrafast spectroscopists in three different continents," he says. "Wherever there was an ultrafast lab in the world, I found it."

Sancar continued studying other DNA repair pathways. Having answered some key questions about excision repair in *E. coli*, Sancar turned his attention to excision repair in humans. Using a strategy that took nearly five years to work

out, Sancar showed in 1992 that humans excise thymine dimers by the same mechanism as *E. coli* (15). "This finding was especially significant since, unlike all other repair mechanisms, the genes for excision repair are not conserved between *E. coli* and humans, indicating this is a convergent mechanism," he says. With the help of an *in vitro* system developed by his postdoctoral fellow, Christopher Selby, Sancar also managed to uncover the molecular mechanism behind the phenomenon of transcription-coupled repair, whereby transcribed DNA is repaired at a faster rate than nontranscribed DNA (16). "I consider this paper one of the most aesthetically pleasing ones of my career," says Sancar. "It employed both classic and modern methods, the data are unambiquous and of high quality, and every experiment worked as predicted by the hypothesis."

Circadian Clock Watching

In 1995, the biotechnology company Human Genome Sciences (HGS, Rockville, MD) released a set of human expressed sequence tags (ESTs) that included an entry for a photolyase ortholog. This sequence proved a bit disconcerting to Sancar, because, as far as he knew, humans did not possess photolyase. "Photolyase has an unusual evolutionary distribution," he says. "For example, *E. coli* has it, but *Bacillus subtilis* does not. At the mammalian level, marsupials have photolyase, but placental mammals, like humans, do not." Two years earlier, Sancar and his group performed sensitive biochemical assays of cells from several organisms to try to detect photolyase but were unable to find it in human samples (17). "So when this EST came out, I said to the postdocs who were involved in the biochemical work, 'Well, maybe we made a mistake, we'd better take a look at this,'" Sancar recalls.

In collaboration with HGS, Sancar's laboratory identified a second human photolyase ortholog as an EST. The next step entailed elucidating the functions of these two orthologs. "Humans use light for two things, seeing in three dimensions and setting their biological clock," says Sancar. Because the receptors involved in sight were well understood, Sancar suspected that these genes worked as circadian photoreceptors. He named them cryptochromes 1 and 2 and generated knockout mice to test his hypothesis. Eliminating either gene produced circadian clock abnormalities, whereas eliminating both completely abolished the clock (18).

"While trying to prove cryptochrome was a photoreceptor, we ended up proving it was an essential component of the circadian clock itself," he says. "This discovery was one of the most exciting things that happened to me, since it opened up an entirely new field to work on." His continued studies with cryptochrome revealed that this field was actually related to other areas Sancar worked on. "I no longer have three separate projects looking at circadian clocks, cell cycle checkpoints, and DNA repair," he says, "but rather I am shifting to having one big project that looks at the interconnectedness of all three areas."

As new research avenues open up to Sancar, he has closed one chapter of his research with the publication of his PNAS Inaugural Article, explaining the photolyase photocycle (1). Nearly 20 years after he first proposed the reaction mechanism for photolyase, instrumentation has improved to a point where the mechanism can be demonstrated. Along with Dongping Zhong and colleagues at Ohio State University (Columbus, OH), Sancar captured the excited flavin intermediate and observed the photolyase photocycle, which involves electron transfer from the flavin to the thymine dimer in 170 ps and then back again from the repaired thymine in 560 ps. With his eye on the circadian clock, Sancar says, "These results represent a partial closing of my 33-year journey on photolyase."

Nick Zagorski, *Science Writer*

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