PNAS PROFILE

Profile of Ta-Yuan Chang

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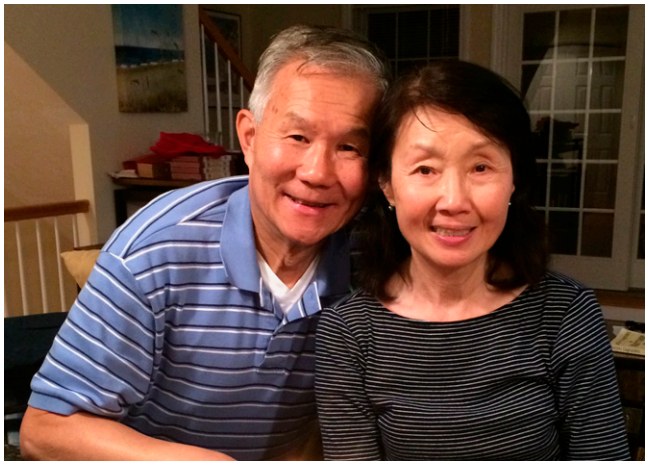
"The most important discovery that I have ever made in my life is that my wife Cathy is a multitalented scientist," says Ta-Yuan Chang, who was elected to the National Academy of Sciences in 2021 and is a professor of biochemistry and cell biology at the Dartmouth Geisel School of Medicine. Chang and his wife, together, lead a laboratory at Dartmouth that over four decades has advanced basic and translational research on cellular cholesterol metabolism, a process that plays a central role in health and disease. Notably, in 1993 the team identified the gene that encodes the cholesterol storage enzyme acyl-coenzyme A:cholesterol acyltransferase 1 (ACAT1). ACAT1 is now a promising drug target for the treatment of Alzheimer's and other diseases. Chang's Inaugural Article (1) demonstrates that inactivating the *Acat1* gene in a mouse model of Niemann-Pick type C1 (NPC1) disease prolongs lifespan. The proposed mechanism could aid the development of disease-mitigating ACAT1 inhibitors.



Ta-Yuan Chang and Cathy Chang. Image credit: Ellen Chang (photographer).

From Opera to Biochemistry

Chang was born in China and raised in Taiwan. His mother was a violinist and his maternal grandfather was the principal of a music school. Chang exhibited early musical talent. When he attended the prestigious Affiliated Senior High School of National Taiwan Normal University in Taipei, his music teacher noticed his potential as a bass singer. She gave him free classical singing lessons, and within a few years Chang was an accomplished amateur singer. He says, "This experience helped me to develop enormous self-confidence." Chang's high school chemistry teacher was another important early mentor. He says, "She made me believe that to do well in chemistry, one does not have to be a genius."

At the National Taiwan University, Chang continued to study music and planned to become a professional singer. However, after listening to the operatic bass Ezio Pinza on the radio and realizing he could never reach that level of excellence in music, he decided to change course. He focused on chemistry and earned a bachelor's degree in 1967.

Somatic Cell Genetic Approach for Cholesterol Metabolism

For his graduate and doctoral studies, Chang traveled to the United States in 1968 to attend the University of North Carolina at Chapel Hill, where his PhD advisor was biochemist Mary Ellen Jones. She encouraged him to focus on the biochemistry of enzymes and to consider an independent research career in a major US university.

Upon earning a doctorate in 1973, Chang became a postdoctoral fellow for 2 years at Washington University School of Medicine, under the mentorship of biochemist P. Roy Vagelos. In 1975, Vagelos joined the pharmaceutical company Merck, and to continue training with Vagelos, Chang spent a year at Merck. "Roy is a great mentor," Chang says. "Six of Roy's disciples went on to become National Academy members. He has tremendous scientific vision and taught us to stick to basic science but to conduct research in biomedically important areas."

Vagelos' research focus was lipid metabolism and membrane biology. Chang proposed a new approach to study lipids by isolating lipid-requiring mutants from mammalian cells, instead of the more commonly used bacterial or yeast cells. Vagelos approved of the approach and advised Chang to read the work of mammalian cell geneticist Theodore Puck, who established the Chinese hamster ovary (CHO) cell line to study somatic cell genetics. In 1977, Chang led a team from the Vagelos laboratory and reported the isolation of the first CHO cell mutant requiring cholesterol for growth (2), demonstrating that specific cholesterol mutants could be selected from mutagenized mammalian cells.

Early Research on ACAT

In 1976, Chang accepted an assistant professor position in the department of biochemistry at Dartmouth Medical School. His wife Cathy joined his laboratory 4 years later. Chang became a full professor in 1988 and served as Chair of the department from 2000 to 2008. Cathy moved up the ranks and became a principal research scientist in 2011.

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The first graduate student to work in the Chang laboratory was Gary Doolittle, now an anesthesiologist in Olympia, Washington. Doolittle initiated the laboratory's research on ACAT, a term that is now more specifically applied to two distinct enzymes: ACAT1 and ACAT2. ACATs are membrane-bound enzymes, and their activity was first described by biochemist Roslyn Alfin-Slater in 1958. Yet, for many years thereafter, it was believed there was just one such enzyme, and its molecular identity remained unknown. ACATs require detergents for biochemical purification, but most detergents inactivate them. Doolittle found that the detergent deoxycholate allowed him to solubilize ACAT from pig liver homogenates without losing activity. He partially purified the enzyme and determined that it may be allosterically regulated by its own substrate, cholesterol (3).

Four Classes of Cholesterol Mutants

In addition to studying ACAT, Chang and his team continued to apply somatic cell genetics to the investigation of cholesterol metabolism. In 1978, he and colleagues isolated the CHO cell mutant M1 (4). Chang says, "M1 provided the first genetic evidence that both cholesterol biosynthesis and unsaturated fatty acid biosynthesis may be controlled by the same unknown regulatory factors." Chang's interpretation was strengthened 2 years later by the isolation and characterization of another CHO cell mutant, 25RA (5). While some peers questioned Chang's interpretation, biochemists Michael Brown and Joseph Goldstein, who together won the 1985 Nobel Prize in Physiology or Medicine for their work on cholesterol metabolism, provided encouragement and support.

Chang shared 25RA with Brown, Goldstein, and their team, who in 1996 used 25RA as a tool to identify a gene, *SCAP*, involved in controlling both cholesterol and fatty acid biosynthesis. Collaborating with Brown, Goldstein, and colleagues, Chang's graduate student Mazahir Hasan, now a professor at the Achucarro Basque Center for Neuroscience in Spain, isolated CHO cell mutant M19 (6). Chang says, "Hasan delivered normal human DNA into M19 cells and showed that certain human genes can correct the M19 mutant defect." The Brown–Goldstein laboratory used these cells as tools to identify a single human gene, *S2P*, which proved to be the second regulatory factor critical to the control of both cholesterol and fatty acid biosynthesis.

Chang's graduate student Kenneth Cadigan, now a professor at the University of Michigan, led work leading to the isolation of the CHO cell mutants CT52 and CT60 (7). These CHO cell mutants had impaired intracellular cholesterol trafficking. Seven years later, Chang shared CT52 and CT60 with NIH biochemist Peter Pentchev and colleagues, who used CT60 as a tool to identify the gene *NPC1*, which, when mutated, can cause NPC disease. Chang's team later reported biochemical evidence that NPC1 is a cholesterol-binding protein (8).

Radical Approach to Study ACAT

When Doolittle left Chang's laboratory in 1981, Chang and his wife, along with a few undergraduate students, attempted to further purify ACAT to homogeneity. At first, they failed. Chang says, "Despite frustrations, we [did] not give up. [Molecular biologist] Har Gobind Khorana [a mentor and supporter of Chang's work] told me, 'You must continue,' and we did."

The team decided on a radical approach: to identify the ACAT gene without purifying the enzyme. They planned three steps. The first was to isolate a mutant CHO cell line that lacks ACAT enzyme activity. The second was to introduce via transfection a large pool of human DNA and isolate stable cell populations that regained ACAT enzyme activity. The final step was to use these cells as a tool to fish out the single human DNA that encodes the *ACAT* gene. Cadigan took the lead on the ambitious plan and isolated the first known cell mutant (AC29) lacking ACAT enzyme activity (9). He and Cathy Chang then proceeded to accomplish the second step (10).

Identification of *ACAT1*

In 1989, Cadigan successfully defended his doctoral thesis and went on to postdoctoral training at the University of Basel in Switzerland and Stanford University. Chang's wife then took the reins of the ACAT project, spending 4 years tenaciously implementing the plan's final step to identify the gene. In 1993, she spearheaded the identification of the full-length complementary DNA with the gene *ACAT1* and showed that it corrects for the ACAT enzyme defects of the AC29 mutant cells (11). Chang says, "This work opened the molecular era for ACAT1 and its relatives in the membrane bound O-acyltransferase (MBOAT) family." For this long-elusive breakthrough, Chang received an NIH MERIT Award in 1994.

In 1998, Chang's team, again led by his wife, purified ACAT1 to homogeneity (12), making ACAT1 the first member of the family to be purified. Three different teams that year identified *ACAT2* as a member of this family. In 2000, Chang and his colleagues demonstrated the quantitative importance of ACAT1 and ACAT2 in the human liver and small intestines (13). In 2005, Chang's team created a detailed ACAT1 membrane topology model (14).

ACAT1 Inhibition and Disease

Over the last decade, Chang's team has used genetic tools and small-molecule *ACAT1* inhibitors to block cholesterol storage with the objective of alleviating neurological diseases. In 2010 they provided genetic evidence that inactivating ACAT1 has beneficial effects in a mouse model of Alzheimer's disease (15). Additionally, they found that inhibiting *ACAT1* in phagocytes in the mouse brain increases the immune cells' ability to degrade proteinaceous toxins, including proteins implicated in Alzheimer's disease (16).

Chang's Inaugural Article (1) provides genetic evidence that inactivating ACAT1 has therapeutic benefits in a mouse model of NPC disease, facilitating cholesterol utilization in the brain, liver, and spleen. Chang says, "It supports the hypothesis that, under certain disease conditions, preventing cholesterol from storage can increase its usage to fight diseases."

Chang says, "Cathy and I would like to see that the discoveries we made in our lab could help to produce a

medicine to alleviate human suffering." In terms of ACAT1 inhibitors, one longstanding challenge has been finding a compound that can cross the blood–brain barrier. His team has identified such a compound, but the findings are preliminary. He says, "The pros and cons of *ACAT1* inhibitors to treat diseases must be evaluated carefully. It may take a long time to determine if any *ACAT1* inhibitor can become a medicine. If Cathy and I can help to accomplish this goal, we may want to celebrate by joining a chorus or taking vocal music lessons together."

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