Video Article

The 2009 Lindau Nobel Laureate Meeting: Roger Y. Tsien, Chemistry 2008

Roger Y. Tsien[1]
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Abstract

American biochemist Roger Tsien shared the 2008 Nobel Prize in Chemistry with Martin Chalfie and Osamu Shimomura for their discovery and development of the Green Fluorescent Protein (GFP). Tsien, who was born in New York in 1952 and grew up in Livingston New Jersey, began to experiment in the basement of the family home at a young age. From growing silica gardens of colorful crystallized metal salts to attempting to synthesize aspirin, these early experiments fueled what would become Tsien's lifelong interest in chemistry and colors.

Tsien's first official laboratory experience was an NSF-supported summer research program in which he used infrared spectroscopy to examine how metals bind to thiocyanate, for which he was awarded a $10,000 scholarship in the Westinghouse Science Talent Search. Following graduation from Harvard in 1972, Tsien attended Cambridge University in England under a Marshall Scholarship. There he learned organic chemistry --a subject he'd hated as an undergraduate-- and looked for a way to synthesize dyes for imaging neuronal activity, generating BAPTA based optical calcium indicator dyes.

Following the completion of his postdoctoral training at Cambridge in 1982, Tsien accepted a faculty position at the University of California, Berkeley. There he and colleagues developed and improved numerous small molecule indicators, including indicators fura-2 and indo-1.

In 1989, Tsien moved his laboratory to the University of California at San Diego, where he and his colleagues developed the enhanced mutant of GFP as a way to devise a cyclic AMP (cAMP) sensor for use in live cells. They initially engineered molecules to take advantage of the conformational change that occurs when cAMP binds to protein kinase A (PKA). By labeling one part of PKA with fluoroscein and another with a rhodamine, they hoped to detect Fluorescence Resonance Energy Transfer (FRET), which would occur when the two molecules were in close proximity. The initial experiments presented numerous difficulties due to the challenges of expressing PKA subunits in *E. coli*, labeling the protein without destroying its function, and delivering the protein to cells via microinjection.

Eventually, Tsien sought a more elegant approach, hoping to use and modify a naturally fluorescent protein that could be expressed in the cell. GFP originally described by Davenport in 1955, extracted and purified by Shimomura in 1965, and cloned by Prasher in 1992 was an appealing candidate. To make the protein more useful for their FRET studies, Tsien and colleagues modified the amino acid structure of the protein (S65T). The improved protein had an excitation peak near that of fluoroscein, and was photostable. Tsien and colleagues also solved the protein's crystal structure, enabling them to generate additional colors with spectral properties suitable for FRET. However, when they attempted to use the GFP proteins in the detection of cAMP, they experienced further difficulties with PKA. Instead, their first successful use of GFP derivatives for FRET was in the detection of intracellular calcium using their engineered calmodulin-based calcium indicator, Cameleon.

In a short time, Tsien's work has led to further technological developments and important scientific findings. GFP and its derivatives have been used in a wide range of biological applications, from the study of protein localization to understanding how HIV spreads from cell to cell. The need for such probes is highlighted by the abundance of research conducted using these fluorescent proteins, as well as the continued development of similar fluorescent proteins, such as the coral-derived dsRED.

Tsien is currently developing genetically encoded Infrared Fluorescent Proteins (IFPs), which with their long emission wavelengths of >700 nm, have the ability to pass through living tissue and improve imaging in living organisms. He is also building synthetic molecules for use in humans. He cites team effort and the contributions of students and post-docs as key components of progress and success: "Even if I had the time, I couldn't have done the experiments, because I don't know how. It's very much a team effort."

Video Link

The video component of this article can be found at https://www.jove.com/video/1575/

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