OBITUARY

Cecile Pickart 1954–2006

Arthur Haas

Cecile M. Pickart died on Wednesday, 5 April 2006, after a courageous fight against kidney cancer. Cecile is best remembered for her seminal contributions to the field of ubiquitin-dependent signaling, a discipline whose history spanned her professional career. Cecile had an incisive intellect and great scientific insight. Her impeccable integrity and quiet grace reflected an earlier age in which science was pursued for the sheer intellectual satisfaction of discovery rather than the attainment of glory or wealth. Although blessed with a sharp wit, she remained to the end a very private person—so much so that few who knew her well were aware of the true extent of her illness, lest attention be drawn to her.

Born in Cheverly, Maryland and later raised in Setauket, New York and Brookeville, Maryland, Cecile graduated *summa cum laude* and Phi Beta Kappa from Furman University in 1976 with a degree in biology. Her love of science was influenced in part by her father, a solid-state physicist, as well as her innate curiosity. Upon graduation, she immediately entered the biochemistry graduate program at Brandeis University under the mentorship of William Jencks, an internationally acclaimed mechanistic organic chemist and enzymologist whose laboratory has trained a series of successful and highly influential scientists. Cecile's formal dissertation work focused on the mechanistic details of the sarcoplasmic reticulum calcium-transporting ATPase. One paper in particular illustrates the intellectual influence of Professor Jencks and Cecile's emphasis on rigorous experimentation and reasoning from first principles, which would mark her later work. Her classic thermodynamic arguments provided a model for the 'how' of calcium transport as well as an underlying explanation for the 'why' of the catalytic mechanism[1] (references herein have been selected to illustrate some of Cecile's most significant papers, in the author's view, as well as the scope of her creativity and insight). However, her first paper as a graduate student in the Jencks laboratory described earlier work on sulfur reactivity with hydroxamic acid in the mechanism of succinyl CoA transferase. This otherwise obscure paper would have an important but unanticipated influence on the direction of her later career.

In the winter of 1982, Cecile joined the laboratory of Irwin 'Ernie' Rose at the Fox Chase Cancer Center as a US National Institutes of Health postdoctoral fellow. The previous three years had witnessed an extraordinary level of productivity, as Ernie Rose collaborated with Avram Hershko and Aaron Ciechanover of the Technion Institute to tease out fundamental aspects of ubiquitin-dependent protein degradation, for which they would share the 2004 Nobel Prize in chemistry. Keith Wilkinson and I had earlier identified the APF-1 protein that became conjugated to targets to mark them for degradation as ubiquitin. With Keith's departure, I was completing the mechanistic characterization of the E1 ubiquitin-activating enzyme. Ernie originally suggested a conventional enzymological question for Cecile that would take good advantage of her strong training in chemistry; however, Cecile was uninterested in "a problem with no scope." Instead, she was immediately attracted to the second component of the ubiquitin-ligation cascade, the ubiquitin carrier protein, E2. E2 was largely uncharacterized, although earlier work made it clear that its catalytic cycle involved sulfur chemistry, with which she was familiar from her earlier paper as a graduate student. Much later, Ernie would comment that, even then, Cecile had a "sense for problems worth working on." She quickly established the basis for transthiolation of E2 by the E1 ternary complex, an essential step for charging the carrier protein, and showed that the 'E2' isolated by covalent ubiquitin affinity chromatography represented a family of similar but distinct proteins. From a chance observation that an enzyme with ubiquitin esterase activity copurified with E1, she again drew on her knowledge of sulfur chemistry to describe the mechanism of the first ubiquitin C-terminal hydrolase (UCH), work that represented a powerful synthesis of chemistry and enzymology[2]. Although Cecile characteristically worked long hours in the lab, she made time for her two passions: singing in a local choral group and playing her cello in a quartet, a talent with which she had worked her way through Furman as a classical musician.

Cecile accepted her first academic post as Assistant Professor of Biochemistry in the School of Medicine at the State University of New York, Buffalo in 1985, and there she continued her work on ubiquitin carrier proteins. Soon, Cecile and then graduate student Zhijian Chen identified and subsequently cloned $E2_{25K}$, a ubiquitin carrier protein with the remarkable property of catalyzing the assembly of free Lys48-linked polyubiquitin chains[3,4]. Two years earlier, Alex Varshavsky's group, in collaboration with Vince Chau, had identified such chains as the degradation signal recognized by the 26S proteasome. Cecile immediately saw that she now possessed a powerful tool for investigating proteasome function, which she exploited in a series of important papers over the next decade that elevated her to an authority on polyubiquitin-chain chemistry. The ability to generate reagent quantities of polyubiquitin chains inevitably led to their crystallization and structural determination, in a collaboration with Bill Cook and his colleagues at the University of Alabama, Birmingham. This important paper provided the first insights into potential mechanisms for formation and recognition of ubiquitin chains by the proteasome[5]. With Quinn Deveraux and Marty Rechsteiner at the University of Utah, Cecile also enlisted polyubiquitin chains to identify the S5a subunit of the 19S regulatory complex as the 26S proteasome subunit responsible for recognition of Lys48-linked degradation signals. The S5a protein would later prove to be the first member of a large and important superfamily of ubiquitin-associated domain proteins[6].

As the pace of Cecile's investigations quickened, her groundbreaking work was increasingly being recognized. After a sabbatical with Dan Finley in the Department of Cell Biology at Harvard Medical School to hone her molecular biology skills, Cecile moved to the Bloomberg School of Public Health at Johns Hopkins University as Professor in their Department of Biochemistry and Molecular Biology. She would hold this position until her death, in a period marking her most productive work.

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Identification of the S5a subunit led to a productive series of papers in which Cecile continued her collaboration with Marty Rechsteiner to characterize the interaction of S5a with Lys48-linked polyubiquitin chains. Point mutagenesis of ubiquitin coupled with quantitative binding studies provided the first insights into the binding determinants for polyubiquitin-chain recognition by S5a, determinants that in part drive the extreme sequence conservation of ubiquitin. Accurate estimates for the affinity of chain association with S5a defined tetraubiquitin as the minimum recognition signal required for degradation and provided the first evidence that such chains represent high-affinity binding determinants for proteasome recognition. Cecile recalled that physically linked binding determinants, as found with polyubiquitin, have affinities significantly greater than the sum of binding energies for individual determinants, due to the entropic loss associated with restricting rotational and translation freedom, a prediction from thermodynamics made by M.I. Page and Jencks to account for proximity effects in enzyme catalysis five years before Cecile entered Brandeis. This synergy results in the remarkable ability of the proteasome to discriminate tetraubiquitin from smaller chains and monoubiquitinated adducts.

Identification of noncanonical polyubiquitin chains linked through other lysine residues required unique subunit packing distinct from the structure first determined for Lys48-linked tetraubiquitin. Cecile and her students began examining the assembly of such noncanonical chains and identified enzymes capable of creating Lys29 and Lys63 linkages[7,8]. The Lys63-specific ligases were particularly compelling, because they function in regulatory rather than target signaling. This insight shifted Cecile's focus to the unique Mms2 E2 variant and its role in DNA-damage repair as well as to the linkage-specific ubiquitin-associated domains of HHR23A[9,10]. Cecile's contributions elucidating the function of ubiquitin in DNA repair brought the field and, fittingly, her career full circle, as this process was one of the first roles identified for ubiquitin conjugation.

In one of the last papers before her death[11], Cecile and Min Wang began to resolve one of the most compelling questions in the field, that of how ligases assemble polyubiquitin chains. As is typical of much of her work, the answer is more elegant than earlier speculations and assumptions. One can only imagine what other wonders Cecile would have discovered in the future. Of certainty is that those of us privileged to know her well are richer for the experience, and we are all diminished by her passing.

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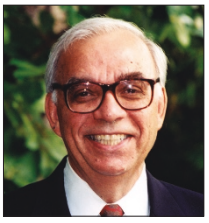

Nicholas Cozzarelli 1938–2006

James C Wang

Nicholas R. Cozzarelli passed away on March 19 of this year, a week before his 68th birthday. Nick was a gifted and passionate scientist and a devoted educator. Since his death, some earlier events in his life have already been reported in the *Los Angeles Times* and in obituaries that have appeared in scientific journals including *ACS Chemical Biology*, *Cell* and the *Proceedings of the National Academy of Sciences of the USA*: that Nick grew up in New Jersey in a poor immigrant family from southern Italy, that Nick's father was determined to give his son an education that he himself never had, and that after graduating from Princeton Nick spent a year at Yale Medical School before joining the laboratory of Edmund C. C. Lin at Harvard Medical School. Some of Nick's many contributions in the later part of his life have also been touched upon in those articles, and his efforts in revitalizing and transforming the *Proceedings of the National Academy of Sciences*, as its Editor-in-Chief in the last eleven years of his life, have been detailed in an "In memoriam" article in the 18 April 2006 issue of the journal[1].

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I knew Nick for nearly four decades. In the 1960s, tremendous progress was being made in the study of enzymes that replicate DNA. After receiving his PhD in 1966, Nick decided to join Arthur Kornberg's laboratory at Stanford University, a Mecca in the world of DNA enzymology. It was there that I first met him. I was at that time measuring the extent of supercoiling of *Escherichia coli* plasmids of different sizes, hoping that such data might provide a clue as to why DNA rings purified from cells were supercoiled. After hearing a talk I gave at Stanford, Nick came up and offered me a sample of an *E. coli* plasmid that he had just discovered, which, with a contour length of a little under 2 μm, was the smallest DNA ring known at that time. The small size and low copy number per cell made it difficult to obtain enough of the plasmid for quantitative measurements; a few days later, however, Nick showed up in my office at Berkeley, with an ice bucket containing a generous sample of the precious DNA. Nick's enthusiasm for science, and his generosity with his time, material and research findings, would remain unchanged over the decades I knew him.

Nick joined the faculty of the University of Chicago in 1968. Because studies on DNA replication in most of the leading centers then were sharply focused on the *E. coli* system, Nick chose to concentrate his own efforts on the DNA polymerases of the Gram-positive bacterium