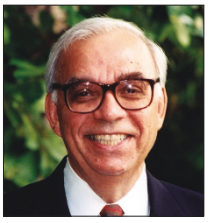
Identification of the S5a subunit led to a productive series of papers in which Cecile continued her collaboration with Marty Rechsteiner to characterize the interaction of S5a with Lys48-linked polyubiquitin chains. Point mutagenesis of ubiquitin coupled with quantitative binding studies provided the 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.

1. Pickart, C.M. & Jencks, W.P. *J. Biol. Chem.* **259**, 1629–1643 (1984).
2. Pickart, C.M. & Rose, I.A. *J. Biol. Chem.* **261**, 10210–10217 (1986).
3. Chen, Z. & Pickart, C.M. *J. Biol. Chem.* **265**, 21835–21842 (1990).
4. Chen, Z.J., Niles, E.G. & Pickart, C.M. *J. Biol. Chem.* **266**, 15698–15704 (1991).
5. Cook, W.J., Jeffrey, L.C., Kasperek, E. & Pickart, C.M. *J. Mol. Biol.* **236**, 601–609 (1994).
6. Deveraux, Q., Ustrell, V., Pickart, C. & Rechsteiner, M. *J. Biol. Chem.* **269**, 7059–7061 (1994).
7. Hofmann, R.M. & Pickart, C.M. *Cell* **96**, 645–653 (1999).
8. Mastrandrea, L.D., You, J., Niles, E.G. & Pickart, C.M. *J. Biol. Chem.* **274**, 27299–27306 (1999).
9. Raasi, S., Orlov, I., Fleming, K.G. & Pickart, C.M. *J. Mol. Biol.* **341**, 1367–1379 (2004).
10. Tsui, C., Raguraj, A. & Pickart, C.M. *J. Biol. Chem.* **280**, 19829–19835 (2005).
11. Wang, M. & Pickart, C.M. *EMBO J.* **24**, 4324–4333 (2005).



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].

The author is the Mallinckrodt Professor of Biochemistry and Molecular Biology, emeritus, at Harvard University.
e-mail: jcwang@fas.harvard.edu

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

Bacillus subtilis, which is far removed from the Gram-negative *E. coli*. One of his early projects was a study of the compound 6-(*p*-hydroxyphenylazo)-uracil, an inhibitor of DNA synthesis in *B. subtilis* but not in *E. coli*. He and his associates soon deduced that the target of this compound is DNA polymerase III and that inhibition involves the formation of a ternary complex consisting of the antimicrobial agent, the polymerase and the DNA template at a position where a C awaits the incorporation of a dGTP.

It was his interest in inhibitors of DNA synthesis that led to a dramatic switch of his research emphasis in the mid-1970s. Nick wrote a review on DNA synthesis inhibitors for the 1977 issue of the *Annual Review of Biochemistry*[2]. Among the numerous inhibitors covered in his review, some were compounds on which his laboratory had previously carried out extensive studies; of the others, two would soon take center stage: novobiocin and nalidixic acid.

In his review, Nick wrote about novobiocin and its more potent analog coumermycin: "Recently a breakthrough has been made on the mechanism of the drugs. An enzyme named DNA gyrase has been isolated from *E. coli*; it has the novel activity of introducing negative supercoiling into relaxed, closed circular DNA." He also wrote about some yet unpublished results on nalidixic acid, an antibiotic still in wide use in the treatment of urinary infections, and gave a long list of enzymes that could be ruled out as its target. It must have crossed his mind that perhaps the newly discovered DNA gyrase might be the target of nalidixic acid as well as novobiocin.

The discovery of DNA gyrase in 1976 by Martin Gellert, Kiyoshi Mizuuchi and their coworkers[3] was a milestone in the study of DNA topology and DNA topoisomerases. Although the term 'topoisomerase' was not coined until 1979, enzymes that transiently break DNA strands to alter the topology of DNA rings, and presumably the topology of DNA loops in chromosomes, had been known since 1971. But DNA gyrase was the first described enzyme with the striking property of converting a relaxed DNA ring to a negatively supercoiled form in an ATP-dependent reaction; thus, the discovery of gyrase gave DNA supercoiling a much-needed boost in biological significance. It was also amazing that the discovery of gyrase was almost immediately followed by its identification as the target of novobiocin and coumermycin; the gene responsible for resistance to these compounds had been known years earlier, but the molecular target of the compounds had remained elusive before the discovery of gyrase.

Compared to novobiocin, nalidixic acid (or nal for short) seemed to be a much more interesting drug. Nal not only was clinically important, it also had been well known since 1969 for its sensitivity-dominance: that is, if a bacterium expresses both a wild-type nal-sensitive protein and a mutant nal-resistant protein, sensitivity wins out and the bacterium will die when exposed to the antibiotic. Could DNA gyrase be the target of nal as well? And, if so, why this sensitivity-dominance?

Like many other scientists, Nick was fiercely competitive. Knowing that Gellert's laboratory would surely test the action of nal on the enzyme they had just discovered did not deter him from his quest for the molecular target of nal. He and his associates had already been purifying this target protein, using a rather tedious assay developed by others a few years earlier. Near the end of 1977, in two back-to-back papers in the *Proceedings of the National Academy of Sciences*, both the Cozzarelli and Gellert laboratories concluded that DNA gyrase was indeed the target of the nal class of antibiotics[4,5].

Two years later, in a paper that received scant attention, Nick and his graduate student Kenneth Kreuzer reported their study of the effect of nal on phage T7 growth in *E. coli* cells bearing a wild-type *nalA* gene or a thermally sensitive *nalA43* gene[6] (the *nalA* gene would later be renamed *gyrA*, when it was firmly established that the gene encodes one of the two DNA gyrase subunits). Kreuzer and Cozzarelli found that T7 phage growth was blocked by nal but not by thermal inactivation of the *nalA43* gene product; furthermore, the inhibition of T7 growth by nal was also eliminated by thermal inactivation of the *nalA43* gene product. In the Discussion section of that paper, they described their interpretation of their results: "…. a poison is formed upon interaction of nalidixic acid with the *nalA* gene product, and thermal inactivation of the *nalA43* gene product eliminates the drug sensitivity of T7 by preventing the formation of this poison." When asked about his proudest achievement as a scientist in a recent interview in the Howard Hughes Medical Institute feature *Ask a Scientist*, Nick answered by referring to his introducing a new perspective on how certain antibiotics work by their conversion of a target enzyme to an intracellular poison[7]. Indeed, in the mid-1980s, Leroy Liu and his associates at Johns Hopkins University Medical School would show that a large number of anticancer therapeutics act by converting DNA topoisomerases to intracellular poisons; the list of quinolone antibiotics in clinical use, of which nal and ciprofloxacin (Cipro) are two examples, would also become longer and longer in the years after 1979.

What is the chemical nature of the nal-induced poison? In their 1977 papers[4,5], both the Cozzarelli and Gellert groups reported a curious product when a DNA-bound gyrase was treated with nal. The antibiotic seemed to induce the formation of a ternary complex, which, when treated with the detergent sodium dodecyl sulfate, would form a double-stranded break in the DNA. Furthermore, a protein appeared to remain associated with the DNA, probably through covalent links to the broken DNA ends. The significance of that observation became clear in 1979. A postdoctoral fellow in Nick's laboratory, N. Patrick Higgins, was working on the cleavage reaction. From discussions among Nick, Higgins and a graduate student in Nick's laboratory, Patrick O. Brown, an idea emerged that the nal-induced cleavage of both DNA strands might reflect a key step in the way DNA gyrase catalyzes its reaction: the enzyme would normally form a transient double-stranded break in the DNA and then pass another double-stranded DNA segment through this break before rejoining it; nal would prevent the rejoining of the broken DNA strands, and hence denaturation of the enzyme holding the broken DNA ends by a detergent would yield the observed linear DNA product. Brown then did a very elegant experiment to show that DNA gyrase indeed acts in that fashion. Such a double-stranded DNA cleavage/passage mechanism was independently deduced by Leroy Liu, while he was a postdoctoral fellow working on a phage T4–encoded DNA topoisomerase in Bruce Alberts' laboratory, from the amazing ability of the T4 enzyme to tie knots into double-stranded DNA rings and to untie them. The *Nature* paper by Liu *et al.* on the T4 DNA topoisomerase[8] and the *Science* paper by Brown and Cozzarelli on DNA gyrase[9] appeared within one month of each other. I vividly recall that both Nick and Leroy would phone and share with me their excitement, often within days, including the results of the key experiments that proved the double-stranded cleavage/passage model. Each was apparently unaware of the other's experiments, and I was prevented by their instructions of strict confidentiality from telling each what I had just learned from the other.

This double-stranded breakage and passage mechanism was novel because the first two DNA topoisomerases discovered, *E. coli* and mouse DNA topoisomerase I (then termed the *E. coli* ω protein and the mouse nicking-closing enzyme, respectively), both act by breaking one DNA strand at a time. Thus, the Brown-Cozzarelli experiment on DNA gyrase[9] and the Liu *et al.* work on T4 DNA topoisomerase[8,10] established a new class of DNA topoisomerases. DNA topoisomerases that transiently break one DNA strand at a time have since been termed the type I DNA topoisomerases, and those of the DNA gyrase/T4 DNA topoisomerase class the type II DNA topoisomerases.

The crucial experiment establishing DNA gyrase and T4 DNA topoisomerase as type II enzymes involved the use of a small

Nick with his daughter Laura Cozzarelli-Wood in 2004.

DNA ring of a particular linking number, Lk, between the two intertwined strands. The double-stranded DNA breakage/passage model predicted that Lk would change by an even number in the absence of relative axial rotation of the two broken DNA ends. Even-number changes in Lk were indeed observed, and even after many cycles of enzyme action, no DNA rings that differed from the original DNA by an odd Lk could be observed. By contrast, the same experiment with mouse DNA topoisomerase I, done in Jerry Vinograd's laboratory four years earlier, showed no such even-number restriction in Lk changes.

Nick's splashing jump into DNA topology was the beginning of his passion on this subject in the next quarter of a century. After his move to the Berkeley campus of the University of California in 1982, the research emphasis of his laboratory shifted to the study of the effects of DNA topology on reactions promoted by various site-specific recombinases, and the molecular and mechanistic information that could be deduced from the observed effects. His keen interest in DNA topology also led him to organize the Program for Mathematics and Molecular Biology, with support from the US National Science Foundation and later the Burroughs Wellcome Fund. The program provided a forum for interchanges among scientists in diverse fields and for introducing very different subjects to graduate students and postdoctoral fellows with widely different backgrounds. Nick also initiated a collaboration with Andrzej Stasiak to apply electron microscopy to determine the precise topology of DNA catenanes and knots. With this powerful experimental tool in hand, he then started collaborations with a number of mathematicians, among them De Witt Sumners and James H. White, to inject mathematical rigor into the problems of enzyme-mediated entanglement of various topological forms of DNA rings. He and his collaborators were able to extract detailed structural and mechanistic information from such topological analyses of the reactions.

In more recent years, excited by the beauty and elegance of 'single-molecule' methods that can manipulate and observe individual macromolecules, Nick started new collaborations with the Paris group of David Bensimon and Vincent Croquette, and then with Carlos Bustamante's group after Carlos' move from the University of Oregon to Berkeley in 1998. Nick's biological insights, and his passion for science, never failed to impress and influence all those who collaborated with him.

Over four decades, Nick and I had many interactions because of our close research interests. I recall sipping wine with him one afternoon in the mid-1970s, in the living room of my Berkeley home, which opened through a long sliding door into a yard covered with white gravel. A large cherry tree was blossoming brightly in a corner of the yard that day, and Nick immediately fell in love with the place and said that some day he would like to find one just like it. Little did we know then that I would soon leave Berkeley for the East Coast, and several years later Nick would move from Chicago to Berkeley and find a dream house with an exquisitely beautiful Japanese garden.

It was always a joy to chat with Nick, whether on a topic on which we shared close views or one on which we vigorously disagreed. Sometimes our debates were about substantive issues, other times about terminology. I remember that once I was telling him my objection to his favorite terms 'strand passage' and 'sign inversion' when used with a mechanistic connotation, because the topology of a DNA ring would dictate that any mechanism for a DNA topoisomerase must involve strand passage and sign inversion, and hence such terms had no mechanistic meaning. Nick gave me a feigned you-hurt-my-feeling look, smiled, and then started his defense with a standard Cozzarelli opening move: "Do you really believe...?" But Nick was always a great sport; he would occasionally concede a point or two, only to return in the next round with a more forceful attack. I had the feeling that Nick relished such debates; at any rate, a deeper friendship seemed to grow out of our disagreements.

The last time I had a long conversation with Nick was nearly two years ago, at a meeting in Santa Fe, New Mexico. We had lunch in a very quiet restaurant and discussed a few issues in the fascinating world of DNA topoisomerases. Even though his lymphoma was exacting a heavy toll on him, he was still carrying on an incredible working load in two cities thousands of miles apart, running a very active research group at Berkeley and serving as the Editor-in-Chief of the *Proceedings of the National Academy of Sciences* in Washington, DC. At the end of our long lunch and discussion, he looked a bit tired. We parted with him saying that he would be walking about in the nearby stores to look for gifts that he might take home. Nick was a tough and critical scientist, but he was also a very warm and considerate person, not only to his family but also to his friends, students and colleagues. Once, we were on assignment at a Midwest university, and a mutual friend invited us to dinner. When Nick and I were on our way to the restaurant, Nick suggested that we should cook up a story so that our very hospitable friend wouldn't insist on footing the bill like he always did. So, near the end of our dinner, Nick told our friend, with a straight face, that for our special assignment the US National Institutes of Health had agreed to reimburse all our travel expenses, and therefore our friend should let the NIH be the dinner host.

Nick phoned me a few months ago to say that he missed answering my calls because his physical condition wouldn't allow him to work long hours and he had to leave for home early in the afternoons. He then inquired about my life in retirement and how my family was doing, and, after a brief discussion about a scientific question that was on his mind, he told me the good news that the chemotherapy he had just endured was apparently successful and that at least the aggressive population of his cancer cells had vanished; he said that he wished that he would soon gain his energy back, so as to devote more time to the research of his laboratory. Neither he nor I knew then that he would soon succumb to the harsh side effects of his therapy. Nick will be very much missed.

1. Nuzzo, R. & Zagorski, N. *Proc. Natl. Acad. Sci. USA* **103**, 6078–6080 (2006).
2. Cozzarelli, N.R. *Annu. Rev. Biochem.* **46**, 641–668 (1977).
3. Gellert, M., Mizuuchi, K., O'Dea, M.H. & Nash, H.A. *Proc. Natl. Acad. Sci. USA* **73**, 3872–3876 (1976).
4. Sugino, A., Peebles, C.L., Kreuzer, K.N. & Cozzarelli, N.R., *Proc. Natl. Acad. Sci. USA*, **74**, 4767–4771 (1977).
5. Gellert, M., Mizuuchi, K., O'Dea, M.H., Itoh, T. & Tomizawa, J.I. *Proc. Natl. Acad. Sci. USA* **74**, 4772–4776 (1977).
6. Kreuzer, K.N. & Cozzarelli, N.R. *J. Bacteriol.* **140**, 424–435 (1979).
7. *Ask a Scientist* <http://www.hhmi.org/askascientist/meet-scientist/scientists/cozzarelli.html> (2006).
8. Liu, L.F., Liu, C.C. & Alberts, B.M. *Nature* **281**, 456–461 (1979).
9. Brown, P.O. & Cozzarelli, N.R. *Science* **206**, 1081–1083 (1979).
10. Liu, L.F., Liu, C.C. & Alberts, B.M. *Cell* **19**, 697–707 (1980).