PAUL TALALAY: THE CATALYST

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ABSTRACT

This chapter outlines the history of the groundbreaking chemopreventive research carried out by Dr. Paul Talalay and colleagues. This research has led to clinical trials examining the ability of Nrf2-activating compounds to ameliorate problems of oxidative stress, inflammation, and environmental pollutants. Such trials are anticipated to have a major impact in decreasing the burden of chronic diseases.

Keywords: broccoli, glucosinolate, isothiocyanate, sulforaphane, phase 2 enzymes

The first era of nutrition research centered on the amelioration of deficiency-related diseases, providing robust, mechanistic insights into the physiological functions of macro- and micronutrients and culminating in a series of evidence-based public health recommendations. In recent decades, however, a new era of nutrition research has emerged from data demonstrating specific molecular roles for non-nutrient, plant-derived dietary compounds that influence cellular signaling and gene expression, thereby providing protection against cellular injury and aging. The proposal of this concept, termed "chemoprotection," signaled a watershed event in nutritional research history, and provided a springboard for many arms of inquiry on bioactive plant-derived compounds. In particular, it led to the understanding of the molecular mechanisms that upregulate cellular cytoprotective proteins.

The impetus for these revolutionary ideas emerged in the mid 1960s, when University of Minnesota researcher Lee Wattenberg published a provocative paper in *Cancer Research* in which he described the beneficial effects of certain compounds on carcinogenesis, challenging earlier paradigms about cancer prevention and raising the possibility that cancer could be thwarted [1]. The ensuing investigations demonstrated that phenolic antioxidants—in particular, the commonly used food preservatives butylated hydroxyanisole, butylated hydroxytoluene, and ethoxyquin—abrogated the carcinogenic effects of a diverse array of structurally unrelated compounds in animal models through enhancement of cells' capacity to increase their intrinsic chemoprotective potential [2].

Wattenberg's work (as well as others') suggested that chemical inhibition of carcinogenesis was achievable: The body's own protective mechanisms were accessible, and could be tapped—even enhanced—to reduce disease risk [3]. Although much of the scientific community understood that human cancers were caused by environmental, nutritional, and exogenous factors, many still considered cancer unavoidable and the feasibility of its prevention far-fetched; nevertheless, Wattenberg's insights spurred a new emphasis on disease prevention.

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A decade later, Paul Talalay, then director of the Department of Pharmacology at the Johns Hopkins University, turned his attention toward mediating the mutagenic effects of pharmaceutical agents, including several antischistosomal drugs. Inspired by (but skeptical about) Wattenberg's observations, Talalay and his colleagues expanded upon Wattenberg's work, focusing their investigations on other antioxidant compounds that might similarly boost cells' intrinsic defensive responses. Earlier investigators had noted that these cellular responses varied, were often non-sequential, and yielded different outcomes, not all of which were favorable—characteristic of the non-linear nature of xenobiotic detoxification, mediated by phase 1 and phase 2 drug-metabolizing enzymes, such as the cytochrome P-450 family of enzymes or the glutathione S-transferases, respectively. Under Talalay's guidance, a multidisciplinary, collaborative approach soon yielded that considerable protection against chemical carcinogenesis could be achieved by induction of these enzymes responsible for carcinogen metabolism [4].

The child of Russian immigrants, Talalay was born in 1923 in Berlin, Germany. When Talalay was 10 years old, he and his family fled Nazi rule, landing first in France, then Belgium. The family eventually settled in London, England, where Talalay attended Bedford School, an all-boys private school whose core values—integrity, responsibility, endeavor, and curiosity—would come to summarize his life and career. The years at Bedford also laid the groundwork for Talalay's future academic success, sparking a profound interest in science. Talalay is one of many illustrious "Old Bedfordians," notable alumni that include British politician Paddy Ashdown and Nobel Prize-winning chemist Archer Martin [5].

Talalay's fortuitous arrival in the United States came in July 1940, just two months prior to commencement of the London Blitz. He continued to pursue his scientific interests, earning his undergraduate degree in biophysics from Massachusetts Institute of Technology and a medical degree from Yale University.

After serving two years as a general surgeon at Massachusetts General Hospital—a brief dalliance in clinical practice that profoundly influenced his research perspective—Talalay began working at the University of Chicago's Ben May Laboratory for Cancer Research. There he focused on the intersection of enzymology, steroid metabolism, and cancer treatment under the mentorship of the late Charles Huggins. In 1963, Talalay became professor and director of the Department of Pharmacology at the Johns Hopkins University. He stepped down from the directorship in 1974 and has remained at Johns Hopkins as the John Jacob Abel Distinguished Service Professor of Pharmacology and Molecular Sciences [3].

Talalay's extensive grounding in enzymology elucidated much of the work that was to come and illuminated the critical physiological roles of the glutathione S-transferases, a family of phase 2 enzymes that drive glutathione conjugation via sulfhydryl groups to an array of endogenous and exogenous electrophilic compounds to facilitate their subsequent detoxification and elimination. Principally, glutathione conjugation serves as the inaugural event in the mercapturic acid pathway and marshals a cascade of events critical in the protection of cellular macromolecules from electrophilic attack and oxidative stress, thus mediating the metabolic inactivation of proximate and ultimate carcinogens or mutagens [4].

Talalay and his colleagues noted that basal concentrations of hepatic and extrahepatic glutathione S-transferases typically are high, upregulate substantially when induced, and demonstrate an absolute requirement for glutathione [6]. Evidence mounted that other phase 2 antioxidant enzymes, including epoxide hydrolase, glucuronosyltransferases, heme oxygenase, and NADPH:quinone oxidoreductase 1

(NQO1), are similarly upregulated. NQO1, in particular, exhibits broad specificity, widespread distribution, and an ability to catalyze obligate two-electron quinone reduction [7]. As such, it demonstrates a significant local protective role in cells as it facilitates glucuronide and sulfate conjugation for subsequent conjugate elimination from the cell. Later work would reveal NQO1's "gatekeeping" roles, including its propensity to bind to and stabilize the tumor suppressor protein p53 against proteasomal degradation, serving a sentinel cytoprotective role [8].

By the early 1980s, Talalay and his colleagues provided evidence that the regulation of phase 1 and phase 2 enzymes was a function of a wide array of seemingly unrelated anticarcinogenic inducers, classified as either bifunctional or monofunctional inducers. The bifunctional inducers (e.g., 2,3,7,8-tetrachlorodibenzo-p-dioxin, polycyclic aromatic hydrocarbons, azo dyes, and naphthoflavone) promote the activities of both phase 1 and phase 2 enzymes, while monofunctional inducers (e.g., diphenols, thiocarbamates, isothiocyanates) stimulate only phase 2 enzymes. Whereas bifunctional inducers tend to be large planar aromatic molecules, monofunctional inducers share little structural concordance [9]. Since phase 1 enzymes promote activation of carcinogens to their more reactive forms, Talalay posited that monofunctional inducers are more promising candidates than bifunctional inducers as anticarcinogens [10].

Soon, three salient characteristics of chemoprotective enzyme inducers emerged: 1) the capacity for monofunctional rather than bifunctional induction; 2) the ability to raise phase 2 enzyme activity in multiple tissues; and 3) low toxicity (as evidenced by their widespread presence in foods or in living matter). This latter feature reduced the need for toxicity testing prior to clinical trials—a notable plus [11]. Quantitative assays already in use in Talalay's lab enabled the identification of a vast array of monofunctional inducers of cytoprotective proteins, including Michael reaction acceptors, diphenols, quinones, isothiocyanates, peroxides, vicinal dimercaptans, heavy metals, arsenicals, and others [12]. Typically, inducers exhibit electrophilicity and thus serve as substrates for glutathione S-transferases. Increased intracellular glutathione levels accompany their induction, enhancing cellular protection [10].

Advances in cell culture techniques led to the development of a simple screening method for rapidly detecting and identifying anticarcinogenic components in human diets [13]. Evidence from epidemiological studies and animal feeding studies had indicated that high consumption of yellow and green vegetables, especially cruciferous vegetables, reduces cancer risk, suggesting vegetables were potential sources of inducer activity [14]. A systematic bioassay of several organically grown vegetables and fruits measured phase 2 enzyme induction, gauged inducer toxicity, and distinguished between monofunctional and bifunctional inducers. The breakthrough discovery came in the early 1990s when Talalay handed then-graduate student Hans Prochaska twenty dollars and sent him on a mission to Baltimore's Northeast Market to buy vegetables. When Prochaska returned to the lab, he began looking for substances in the vegetables that switched on the cells' protective mechanisms [3]. Of all the vegetables assayed, broccoli demonstrated particularly high phase 2-inducer activity. Sulforaphane, an isothiocyanate, was isolated and identified as the predominant chemical entity in broccoli responsible for phase 2 enzyme induction, exhibiting monofunctional inducer activity without phase 1 induction [15, 16].

The discovery that an isothiocyanate was responsible for the anticarcinogenic activity in broccoli generated considerable excitement and interest because it was a dietary component and readily available. Isothiocyanates and their glucosinolate precursors are present in many higher plants, especially among cruciferous vegetables [15]. Furthermore, the chemoprotective effects of isothiocyanates had been clearly demonstrated in rodents, likely due to isothiocyanates' capacity to induce phase 2 enzymes [15]. In addition,

sulforaphane induces apoptosis, and inhibits cell-cycle progression, angiogenesis, and the activities of cytochrome P450s and histone deacetylases, collectively impeding tumor growth [16].

The results of the Talalay group's landmark five-year study, published in 1992 in *Proceedings of the National Academies of Science* (PNAS), hailed by the *New York Times* and *The Congressional Quarterly*, and declared one of the top 100 scientific discoveries of the 20th century by *Popular Mechanics*, was not viewed as a victory initially, and was in fact rejected by at least one notable publication before PNAS' acceptance [2, 3, 17, 18].

But the seminal paper produced a ripple effect that influenced dietary and, subsequently, agricultural practices worldwide. In 1991, the year before the study was published, per capita broccoli consumption in the United States averaged 5.29 pounds. In 2011, per capita consumption averaged 8.45 pounds, a 60 percent increase in two decades. More importantly, the study heralded a paradigm shift in cancer research: the disease once considered inevitable for many was, in fact, preventable, and dietary compounds—such as sulforaphane—could play a key role in the prevention equation.

Further investigation revealed, however, that sulforaphane was an artifact of isolation and was in fact the degradation product of glucoraphanin, a glucosinolate—sulfur-rich precursor molecules characterized by a β-D-glucopyranose moiety, sulfur-linked to a β-thioglucoside-*N*-hydroxysulfate, and an amino acid-derived side chain [19]. Glucosinolates coexist with but are compartmentally segregated from plant myrosinases, endogenous β-thioglucosidase enzymes strategically sequestered throughout plants, whose sole known substrates are glucosinolates. Approximately 120 distinct glucosinolate structures have since been identified [20].

The trajectory of Talalay's research had changed course. The autumn of 1993 ushered in the inauguration of the Brassica Chemoprotection Laboratory at the Johns Hopkins School of Medicine, now the Lewis B. and Dorothy Cullman Chemoprotection Center, and a new era of discovery. Talalay's mission expanded, and the group now sought insights into the mechanisms by which plants develop self-protective chemical agents that can also afford animal cells protection against the damaging processes that lead to chronic disease.

Sulforaphane's isolation in particular facilitated identification of the elusive mechanisms by which antioxidant compounds prevent carcinogenesis. Specifically, tandem, coordinated phase 1 enzyme suppression inhibits ultimate carcinogen formation, while phase 2 enzyme induction detoxifies residual electrophilic metabolites generated by phase 1 enzymes, thereby preventing DNA damage [21]. These highly inducible cytoprotective pathways, which typically function in "idle," escalate in the presence of sulforaphane.

There also came an understanding that many of the genes encoding cytoprotective proteins share common transcriptional regulation through the Keap1-Nrf2-ARE pathway, a key mediator of cytoprotective responses to oxidative and electrophilic stress. Keap1 (Kelch-like ECH-associated protein-1) presents transcription factor Nrf2 (nuclear factor-erythroid 2 p45-related factor-2) for ubiquitination and subsequent proteasomal degradation. Isothiocyanates such as sulforaphane react with highly reactive cysteine thiol groups on Keap1, eliminating the protein's ability to target Nrf2 for degradation. Consequently, Nrf2 amasses and then translocates to the nucleus where it binds to antioxidant response elements (AREs), specific DNA sequences in the upstream regulatory regions of cytoprotective genes.

This cascade of events sets in motion the coordinate transcription of a diverse group of cytoprotective proteins, altering the metabolism and elimination of environmental procarcinogens in humans, and affording protection against the damaging effects of environmental toxins by enhancing their detoxification. Sulforaphane is the most potent naturally occurring small molecule inducer of these cytoprotective proteins.

Talalay's group illuminated the relationship between inducer compounds' chemical structures and their potencies, and, together with the groups of Masayuki Yamamoto and Tom Kensler, identified the key players and events that mediate induction through the Keap1-Nrf2-ARE pathway, providing crucial insights into the molecular mechanisms by which phase 2 enzyme induction occurs. Yet, the impetus for induction remained an enigma. The mystery was solved when Albena Dinkova-Kostova, a young research associate and biochemist in Talalay's lab, noted that specific, highly reactive sulfhydryl groups located between the protein-binding domains of Keap1 likely serve as "cellular sensors" that recognize and interact with inducer compounds, thereby initiating phase 2 enzyme induction [22].

By the late 1990s, Talalay's work with sulforaphane was deeply rooted in basic science and poised to move into clinical trials in humans. But the seasonal and practical aspects of broccoli acquisition had became problematic, so Talalay's team turned to production, which yielded the discovery that young broccoli sprouts contained substantially greater quantities of glucoraphanin than mature plants—a serendipitous event that enabled standardization for future investigations [19].

Production presented its own challenges, however. In a makeshift greenhouse comprised of steel growing racks, plastic tarps, and a portable electric space heater, Talalay colleagues Jed Fahey and Tom Kensler grew broccoli sprouts for a clinical trial to gauge sulforaphane bioavailability in humans. It was the winter of 2003 in rural China, and bitterly cold. The duo watered the sprouts every two hours, risking electrocution, freezing, and flooding. In the end, they produced more than 200 kilograms of broccoli sprouts and one enormous batch of bitter-tasting broccoli sprout "tea." Future trials, which were among the first to "reduce-to-practice" the concept promulgated by Talalay so many years ago, relied on freeze-dried preparations developed in less spartan accommodations [23, 24].

The first trials were simple, proof-of-principle studies that provided both qualitative and quantitative evidence that sulforaphane-mediated enzyme induction, via broccoli-sprout infusion, was protective against cancer and occurred in a dose-dependent fashion [25]. Humans tolerate broccoli sprouts well, and nearly two decades of clinical trials involving broccoli sprouts and spanning a wide range of human disease states, including air pollution toxicity, asthma, autism, chronic obstructive pulmonary disease, radiation dermatitis, schizophrenia, and cancers of the bladder, breast, colon, lung, and prostate, among others, are complete or are currently underway. Nearly 25 years since the isolation and identification of sulforaphane, the research on broccoli, sulforaphane, and other plant-derived bioactive compounds remains robust and thriving. Current aims focus on refining the understanding of the mechanisms by which these compounds reduce chronic disease risk.

A diverse portfolio of activities has been ascribed to sulforaphane, most of which are related to its ability to upregulate cytoprotective proteins. However, in 2002, Jed Fahey heard of anecdotal evidence suggesting broccoli sprouts were effective in treating *Helicobacter pylori* infection, a gram-negative bacterium implicated in the etiology of peptic ulcers and gastric cancer. In the developing world, *Helicobacter pylori* infection rates exceed 90 percent and are associated with a significant cancer burden. Fahey discovered that sulforaphane is bactericidal for *Helicobacter pylori* and reduces gastric tumor

formation in animal models. As such, Fahey's discovery speaks to the potentially immense public health applications for sulforaphane and other plant-derived bioactive compounds.[24, 26, 27]

The Talalay group established (and continues to practice) a legacy of strong basic science applied against the backdrop of traditional medicine, anecdotal evidence, and epidemiological surveillance data, and successfully translated their findings to the clinic. The group's combined intellectual curiosity coupled with their scrupulous techniques likely stand unmatched in any setting.

In 2012, Talalay and colleagues Kensler and Fahey posited a new strategy to combat the escalating global burden of chronic disease: "green chemoprevention." In the near future, chronic disease treatment likely will exceed the healthcare delivery capabilities of many developing nations. However, a robust body of evidence demonstrates the "green" protective effects of isolated phytochemicals in many whole foods and their extracts. Insights into the mechanisms by which these phytochemicals exert their protective effects can be translated to native foods or potentially influence the introduction of other, culturally appropriate, protective foods.

Together, sulforaphane and broccoli sprouts serve as a model for green chemoprevention: Just as broccoli consumption increased in the wake of the overwhelming evidence supporting its chemoprotective effects, perhaps future identification of plant-derived foods with similarly chemoprotective systems that fit into the dietary practices of individual cultures might move populations toward consumption of those foods, especially in the developing world. In this way, green chemoprevention, the implementation of food-based, frugal, and culturally appropriate intervention strategies, will serve rich and poor alike [23, 24].

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