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Mutagens and Multivitamins

Not one to shy away from controversy, Bruce Ames has pitted himself against industry groups, environmentalists, and his peers through his work identifying DNA mutagens. And he's not done yet.

By Megan Scudellari

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On an otherwise ordinary day in 1964, Bruce Ames picked up a box of potato chips and read the list of ingredients. A biochemist at the National Institutes of Health (NIH) in Bethesda, Maryland, Ames spent his days studying mutations in strains of *Salmonella,* so it wasn't unusual that he began to wonder if any of the preservatives or chemicals on that long list of ingredients might mutate DNA. Ames decided to use his *Salmonella* to try to detect genetic damage caused by chemicals. "I figured the world needed some quick, easy test to detect mutagens," says Ames.

Ames had hundreds of strains of *S. typhimurium* with mutations in the genes required to produce histidine, a standard amino acid for making proteins. These strains could not grow without the addition of histidine. When millions of bacteria were placed in media lacking the amino acid, however, a few would spontaneously mutate, produce histidine again, and survive as a colony. Ames figured if he added a chemical, such as a potato-chip preservative, to the *Salmonella* strains, and the chemical increased the number of surviving colonies, it was a mutagen.

"Nutrition is a muddy field, but I like getting into muddy fields."

Ames began tinkering with the test as a hobby. Then, in 1967, when he moved to the University of California, Berkeley, he got some undergraduates to help him develop the assay further. They added rat liver homogenate to the *Salmonell*a as an approximation of mammalian metabolism, which activates many chemicals whose mutagenic action couldn't be detected by the bacteria alone, and the "Ames test" was born—a simple, inexpensive method for detecting DNA mutagens. Ames immediately put the assay to work: he tested 174 suspected cancer-causing chemicals for the ability to mutate DNA, using a range of concentrations up to the level toxic to the bacteria—and a whopping 90 percent of them were mutagens. It was some of the first evidence that carcinogens mutate DNA.

"Like a lot of hobbies, it slowly took over my life," says Ames. Over the years, he grew and gave away thousands of samples of the bacteria to other laboratories, and today the Ames test is still used in academic and industrial laboratories worldwide.

His work on mutagens subsequently drew Ames's interest to cancer prevention, then age-related diseases, then nutrition. A recipient of the National Medal of Science from

President Bill Clinton, Ames has published more than 550 papers and is one of the most-cited scientists across all fields.

Here, Ames takes us on a whirlwind tour of his career, from studies of hair dye and children's pajamas to the defense of pesticide use and vitamins.

AMES THE ACADEMIC

Middling around. Ames attended the Bronx High School of Science in New York City, and then went straight to Cornell University after graduation. "I was never a top student. I was always marching to my own drummer. I'd get interested in Tolstoy and read all his books, then get interested in folk dancing and do a lot of folk dancing, and so on."

Molding students. In 1950, the California Institute of Technology accepted Ames into its PhD program—"despite my less than stellar grades," he admits—where he joined the lab of Herschel Mitchell. Under Mitchell's mentorship, Ames studied the biochemical genetics of mutant strains of the bread mold *Neurospora* that couldn't produce histidine. "No one knew much about the pathway, and Mitchell really encouraged students to just go off on their own, so I worked on it using mutants to figure out the pathway and got my PhD in three years. It gave me confidence in my creativity, even if I was incorrigibly distractible."

***Salmonella* switch.** Ames then did a postdoc at the NIH and stayed on, first as an independent investigator and later section chief, for 14 years. "I continued in biochemical genetics. Phil Hartman at Johns Hopkins was pioneering bacterial genetics in *Salmonella,* and he started isolating histidine-requiring mutants. I soon realized that was an easier system to work on, so I switched to *Salmonella*." In this species, Ames's group found that the histidine genes existed in a cluster, later known as the histidine operon, and were controlled as a group by a regulatory sequence producing a single RNA. It was one of the earliest studies documenting how genes are turned on and off.

To dye for. In 1973, after moving to Berkeley, he published details of the Ames test and put it to use straightaway. "I used to teach an undergraduate lab at Berkeley, and I told my students to go bring something in to test. And you know students, what do they test but marijuana and birth control pills and things like that? But one day a student brought in his girlfriend's hair dye, and it was screamingly mutagenic. So I sent one of my technicians out to the drug store to buy $100 worth of hair dyes. All the permanent hair dyes were mutagenic because they contained aromatic amines, which are often carcinogens. We published a paper on it, and I sent a copy to all the hair-dye companies in the world and said, 'You guys need to start thinking about this.' They eventually developed hair dyes that aren't mutagenic."

Cataloging cancer. Ames's team subsequently showed that cigarette smoke was mutagenic, as was the main flame retardant used in children's pajamas, a chemical called tris-BP. "I didn't want to put my kids in these pajamas, so we bought their pajamas in Europe when we were there," he says. Around the same time, Ames

became interested in animal cancer tests. “I wanted to look at the relationship between mutagens and carcinogens in animal tests. No one had ever systematized that.” Ames applied for a federal grant to create a database of the potency of rodent carcinogens, but was turned down flat. “They said I didn’t know any pathology or statistics and I wasn’t the right person to do it. But we wanted to do it, and no one else was doing it, so we did it.”

Big dose of controversy. Using that database, the still-active Carcinogenic Potency Database, Ames and his collaborator Lois Gold “got into a number of controversies,” he says. “Half of the chemicals tested, whether natural or synthetic, in the standard assay at the maximum tolerated dose were carcinogens. That made us suspicious; something was fishy.” They argued that the huge dose, not the chemical formulation itself, was responsible for inflammation, cell death, and cell proliferation. Their conclusions—that animal cancer tests do not provide a good assessment of low-dose cancer risk—“got all the scientists that had spent their lives doing these [animal cancer] tests angry at us,” says Ames.

Rankling ranking. In 1987, Ames and Gold ranked natural and synthetic pesticides and found that cancer risks from traces of pesticide residues on fruits and vegetables are minuscule compared with the cancer-causing potential of some natural chemicals in plants. “We wrote a review pointing out that every plant has a hundred or so toxic chemicals—nature’s pesticides—to kill off insects, animals, and other predators, and that we were getting 10,000 times more of them than [of] man-made pesticides. Still, everybody is buying expensive organic food,” says Ames. “It’s the new religion. We won the scientific battle but we lost the public-relations battle.” For that work and more, arguing that traces of synthetic chemicals are not a cancer risk, Ames and Gold have been criticized as being in the pocket of the pesticide industry, despite never accepting money, consulting with industry companies, or testifying in trials.

AMES THE ANALYST

Big break. In 1989, cytogeneticist Jim MacGregor spent a sabbatical year in Ames’s lab at Berkeley. “Before he came to the lab, he was looking at radiation breaking chromosomes in precursors of mouse red blood cells, and one day all his control mice were full of chromosome breaks. It turned out the company that sold him the vitamin mix for the mice had left out folic acid, and a lack of folic acid breaks chromosomes just like radiation does. MacGregor started looking in people, and he found a guy who had 20 times the level of chromosome breaks as everyone else. The guy ate a pretty poor diet without a lot of veggies, so he was folic-acid deficient.” Ames was already familiar with folic acid: Herschel Mitchell, Ames’s graduate mentor, discovered folic acid by isolating it from four tons of spinach. “Around 10 percent of the US population and half the poor were folic-acid deficient, so I said, ‘I’m getting into this area.’ Nutrition is a muddy field, but I like getting into muddy fields.” A few years later, Ames and his team determined that folate deficiency results in the massive incorporation of uracil, an RNA chemical base, into DNA, leading to chromosome breaks after excision by a repair enzyme.

Damaging diet. “From there, I became interested in all the essential vitamins and minerals. There are about 30 of them, and according to recommended dietary allowances from the USDA, almost everyone in the population is low in one or more. So I started looking to see if you get DNA damage from a vitamin or mineral deficiency.” Beginning at Berkeley and continuing at Children’s Hospital Oakland Research Institute (CHORI), which Ames joined in 2000, he and his lab found many examples of this phenomenon: iron deficiency causes neuron decay and mitochondrial DNA damage, for example, and zinc deficiency damages DNA and disables tumor suppressor p53. But Ames still didn’t know how such deficiencies could directly damage DNA.

Winners and losers. “Then one day I had this epiphany: When you run out of a vitamin or mineral, which happened all the time throughout evolution, maybe nature rations what it has.” In 2006, Ames proposed the triage theory—that natural selection has developed a rationing response to shortages of micronutrients (vitamins and minerals). When cells run out of a vitamin or mineral, that scarce micronutrient is allotted to proteins essential for short-term survival. Proteins needed for long-term health, including those that protect DNA, lose out and become disabled. With Joyce McCann at CHORI, Ames has demonstrated this rationing is present in cells short on vitamin K and selenium: essential proteins incorporate the micronutrients, and nonessential proteins are disabled or lost. “This suggests you’re paying a price any time you get a little low on any vitamin or mineral,” says Ames.

Nutrients for all. In an effort to apply his nutrition discoveries to cancer prevention, Ames helped develop a micronutrient-dense, low-calorie fruit bar, called the CHORI-bar. “The local USDA was making a bar out of excess California fruit, and we said, ‘Hey, can you add some vitamins to that bar?’ They said sure, and now we’ve been collaborating for about 10 years. It tasted terrible when we first made it, but it’s quite tasty now.” And consumption of the bar is showing an association with indicators of good health. In a two-week clinical trial involving 25 individuals, the addition of two CHORI-bars per day to a typical American diet, with no other dietary interventions, raised levels of high-density lipoprotein and improved antioxidant defense.

Hormonal reaction. Ames’s latest work involves nutrient deficiency and brain dysfunction. “It turns out vitamin D is converted into a hormone that controls 900 genes, many in the brain. I have a wonderful new postdoc, Rhonda Patrick, an energetic scholar, who started looking into autism and vitamin D.” It was previously known that individuals with autism often have low levels of vitamin D in the blood, as well as low serotonin, a hormone critical for social behavior, in the brain. Earlier this year, Patrick found a mechanism linking the two: vitamin D hormone activates the enzyme that converts tryptophan to serotonin in the brain. “This is relevant not only to autism but to antisocial behavior and all kinds of psychiatric disease,” says Ames. “The four papers Rhonda is churning out are as important as any that have ever come out of my lab.”

AMES THE ADVOCATE

The cost of innovation. "American science is really in trouble. Entitlements are eating up the entire federal budget. It's the worst I've seen in all my 60 years in science. If you're innovative, it's the kiss of death. I've been doing most of my innovative work through private charity and [out of] my own pocket."

iVitamins. "I think the future of avoiding the degenerative diseases of aging is not drugs but getting your metabolism and nutrition tuned up. In the future, you will put your finger in a machine and have all the marker proteins in blood analyzed from a finger prick. The machine will say, 'You're low on magnesium' and send a message to your iPhone that you should eat a big plate of spinach or kale every once in a while. Everybody knows their cholesterol number, but in the future they'll know their magnesium number, vitamin D number, and more."

Practice what you preach. "I have an Italian wife, so I get a wonderful Mediterranean diet: lots of fish, vegetables, and fruit for dessert. My wife keeps nagging me to get more exercise, but I told her that when I feel like exercise, I run my experiments, I skip my controls, and I jump to conclusions. After I told that joke about 50 times, she said, 'I've heard enough of that damn joke. I'm getting you a personal trainer.'"

Die laughing. "I'm 85, so I don't know how many more years I have left, as my mitochondria are decaying ever more rapidly. However, I've finally figured out what they're decaying into—hypochondria. But seriously, my five-year plan is to live to 90. My wife says I should have a 10-year plan instead."

GREATEST HITS

- Created the Ames test, an assay to determine if a chemical is mutagenic.
- Demonstrated that most carcinogens are mutagens.
- Built a comprehensive database of carcinogen potency.
- Showed that many nutrient deficiencies cause DNA damage and proposed the triage theory as a mechanism by which this occurs.
- Developed a nutrient-rich fruit bar designed to improve metabolism and prevent age-related disease.
- Uncovered a mechanism linking vitamin D–deficiency to serotonin synthesis, autism, and brain dysfunction.