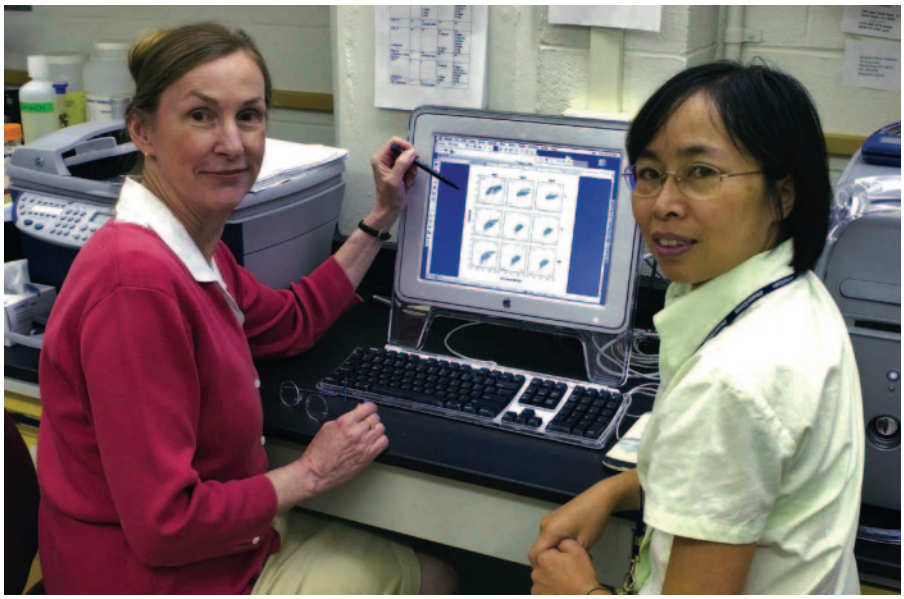
Profile of A. Catharine Ross

Vitamin A's vital role in healthy vision and skin has been well known for decades. Deficiency in vitamin A (retinol) causes the leading form of preventable blindness in children worldwide and can raise the risk of disease and death. What has been less known about vitamin A is its specific, mechanistic role in immune function.

Nutritional biochemist A. Catharine Ross, elected to the National Academy of Sciences in 2003, has spent over 30 years teasing apart the metabolic pathways directing the activities of this micronutrient and the part it plays in the immune response. In her Inaugural Article in this issue of PNAS (1), Ross and colleague Qiuyan Chen explore how retinoic acid, the active metabolite of vitamin A, regulates B cell population dynamics and antibody gene expression.

Ross's research career is particularly unique in that she has investigated biochemical questions about vitamin A within the context of diet and nutrition. Ross's broad appreciation for varying aspects of nutritional science has provided insight and background for her current role as an editor of the *Journal of Nutrition* (from 2004 to present) and during her two terms as a member of the Food and Nutrition Board of the National Academies' Institute of Medicine (1997–2004). This body is charged with specifying the recommended daily dietary allowances, which serve as a nutritional model for many countries.



Ross with coauthor Qiuyan Chen, viewing a supporting information figure from their PNAS article.

California Dreaming

Ross, a native Californian, initially developed an interest in nutrition during her college years. A zoology major at University of California, Davis, Ross enrolled at nearby University of California, Berkeley, for the spring semester of 1969. During her stint at Berkeley, she took an introductory course in nutrition with pioneering nutritional scientist Doris Calloway. Calloway studied the dietary requirements of healthy individuals in a controlled environment and is credited with establishing many requirements of human nutrition through nutritional balance studies.

For Ross, one particularly memorable aspect of Calloway's class was a field trip to a U.S. Department of Agriculture laboratory near Berkeley. "The lab was fascinating, it was an exposure to new technology and new concepts," says Ross. "We saw freeze-dried coffee, which was new at the time. The idea that you could freeze-dry something and then reconstitute it was amazing." Calloway also invited guest speakers to the class, one of whom was "a real biochemist," as Ross puts it. "This introductory course made me see how I could focus things that I liked, biochemistry and physiology, for example, around nutrition, which was a really relevant subject," she says.

Not only coursework whetted Ross's scientific appetite, as life in California primed her for a career in nutrition. "[Nutrition] interested me anyway because I liked foods and cooking and the California lifestyle," she says. Growing up in Napa, CA, Ross was surrounded by food production: "People were working in the vineyards, and everybody was growing things. And I had an aunt who was very interested in what you would now call organic farming. There was just a lot of opportunity to be interested in nutrition."

Cross-Country to Cornell

The semester at Berkeley and her studies with Calloway set Ross's career track. After marrying photographer and artist Alex Ross in 1969 and receiving her zoology degree from the University of California, Davis, in 1970, she and her husband drove cross-country to Cornell University (Ithaca, NY). Ross enrolled in a master's program in the Graduate School of Nutrition and dove into the life of a laboratory scientist. Her exposure to different aspects of nutrition at Cornell became instrumental in throwing a pragmatic spin on her future research. At Cornell, Ross "was trained by renowned bench scientists. I saw how the science was applied and the importance of nutritional science to public policy and human life," she says. "On a daily basis I met with people who were implementing changes in the outside world."

Ross received her master's degree in nutritional science in 1972, with an emphasis on nutritional biochemistry, and began research work with Donald Zilversmit, a professor of nutritional sciences. "I was very fortunate to be chosen by Donald Zilversmit," says Ross. "He was a very rigorous scientist and a creative and independent thinker." Zilversmit's research focused on cholesterol and lipid metabolism. Says Ross, "What I really liked about working on cholesterol and lipoprotein metabolism was that it had an important biological problem behind it, namely atherosclerosis and how lipoproteins contribute to atherosclerosis." Ross's graduate work was her first entry in lipid biochemistry, and though she has since branched off, it remains the foundation of her research to this day.

Zilversmit pioneered the use of radioisotope and chemical tracers. Ross used a radioactive isotope of vitamin A as a physical tracer to study the metabolism of chylomicrons, intestinal lipoproteins that carry lipids from the intestine to the body. "This was my intro to vitamin

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This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 14142.

A," says Ross. "Through reading the literature, I just became intrigued with its biology and its metabolism and have pretty much focused there ever since."

Ross's challenge at the time was to discover the origin of unusual cholesterol-rich lipoproteins that arose in the plasma when rabbits were fed cholesterol. The question stemmed from the notion that if these particles had a dietary origin, then their possible role in atherosclerosis could be minimized by a change in diet. The structure of these droplets did not reveal whether they were produced in the liver or were chylomicron remnants. Ross used radioactive vitamin A, which was packaged into chylomicrons in the intestine but not present in the very low-density lipoprotein droplets secreted by the liver, as a metabolic marker to identify chylomicrons as the source (2).

Protein Partners

In 1976, Ross completed her Ph.D. in the Department of Biochemistry, Molecular and Cell Biology at Cornell and entered the field of nutrition in its heyday. She focused on developing a model to explain how the body regulates vitamin A and its active metabolite, retinoic acid, which functions much like a steroid hormone. "Retinoic acid regulates gene expression, so its concentration is critical," says Ross. "Yet vitamin A is received through the diet, so what physiological mechanisms regulate vitamin A and its conversion to retinoic acid in the body?"

Ross believed the answer must lie in proteins. "Vitamin A is a lipid and can dissolve in membranes and in fat droplets and cells, but it doesn't have a lot of direction to it," she explains. "So it is really the interaction of certain retinoids with proteins that exert control over metabolism." The first such interaction to be identified was that between retinol and its plasma transport protein, retinol-binding protein (RBP), purified by DeWitt Goodman's laboratory at Columbia University (New York) in the late 1960s. Ross joined Goodman's laboratory for postdoctoral studies on vitamin A transport and the interactions of retinoids with binding proteins. The existence of RBP, coupled with work by Frank Chytil's laboratory at Vanderbilt University (Nashville, TN), led Ross and others to believe that RBP must have an intracellular counterpart directing retinol traffic within the cell.

In Goodman's laboratory, Ross used classical protein chemistry in the "grind-and-find era" to purify cellular retinol-binding protein (CRBP-I) and cellular retinoic acid-binding protein (CRABP-I). "These are the molecular trucks that transport retinoids within cells," she says. "That was really one of my first discoveries along the way" (3, 4). Since these findings, researchers have shown that these proteins are members of a much broader fatty-acid-binding protein superfamily.

After completing her postdoctoral research in 1978, Ross struck out on her own, securing an assistant professorship in biochemistry at the Medical College of Pennsylvania (Philadelphia, PA). With a strong desire to find her own niche, Ross began exploring how mammary and hepatic tissues receive vitamin A. From her previous studies, she knew that retinol was the prominent form of vitamin A in plasma. The literature, however, revealed that retinyl ester was the form present in milk and the most abundant stored form of vitamin A in the body. But retinyl esters rarely cross membranes from blood into tissues, such as mammary epithelium. Within the mammary gland, retinyl esters are hydrolyzed and enter the mammary cells as retinol before conversion back into a retinyl ester. "I set out to identify an enzyme in lactating mammary tissue that would be capable of reesterifying retinol," says Ross. Within 2 years, she identified acyl CoA:retinol acyltransferase (ARAT): "I guess I consider myself the mother of ARAT, which was a very important piece of work for me because it established the direction of my research for many years" (5, 6).

"We are doing studies that test the pros and cons, the yin and yang of vitamin A."

During the mid- and late 1980s, while ascending to the ranks of associate professor and then full professor of biochemistry at the Medical College of Pennsylvania, Ross studied ARAT physiology more closely and became skeptical that it was the primary mechanism for converting retinol to retinyl esters for secretion or storage. ARAT explained the production of retinyl esters in the mammary gland but not in the liver. Instead, enzyme studies with CRBP and retinol yielded a new enzyme, lecithin:retinol acyltransferase (LRAT). LRAT transformed retinol to retinyl esters for storage in the liver. Ross later cloned the gene for LRAT from rodent and human liver and demonstrated the regulation of its expression and activity in several tissues (4, 6).

Antibodies Respond

As Ross elucidated the metabolic pathways of vitamin A with the characterizations of ARAT and LRAT, results of landmark public health studies would alter the course of her research. One study conducted in 1983 by Alfred Sommer, of The Johns Hopkins Bloomberg School of Public Health (Baltimore), found that mortality rates were higher in young children who were deficient in vitamin A (7), suggesting that the immune system might be affected. In collaboration with Christopher Taylor, also at the Medical College of Pennsylvania, Ross tested whether vitamin A deficiency reduced antibody responses in young animals. Her hunch proved correct, as antibody responses to some antigens, but not all, were reduced in these animals. A dose of vitamin A, however, yielded normal antibody responses, indicating the effect was reversible (8).

Since this study, Ross has dedicated a part of her research to explore how vitamin A regulates antibody production. The antigen-specific antibody responses led Ross to begin coimmunization experiments in the late 1980s, which continued when she accepted a position as a professor of nutrition at Pennsylvania State University (University Park, PA). She wanted to know whether combining the pseudomonal LPS antigen, which was immunogenic in vitamin A-deficient animals, with the weaker pneumococcal antigen could generate an amplified response to pneumococcal antigen in vitamin A-deficient animals. "I can still remember that afternoon when we did our first experiment because the results were quite dramatic," she says. "The response to the pneumococcal polysaccharide was very, very low in deficient animals, but in animals that were coimmunized with the *Pseudomonas* LPS, the response was quite elevated." This finding implied that the response was not defective but was dysregulated (9). Since then, Ross has gradually shifted her focus from vitamin A, the nutritional form, to its active metabolite, retinoic acid, investigating how retinoic acid modulates antibody production and the immune system.

In her PNAS Inaugural Article (1), Ross investigates the effects of retinoic acid on naïve B cell activity and antibody gene expression. Ross and Chen demonstrate that when a naïve population of B lymphocytes is stimulated to become antibody-producing cells in the presence of retinoic acid, a decrease in B cell proliferation and an increase in immunoglobulin G1 (IgG1) expression

are observed. These findings suggest that retinoic acid attenuates B cell proliferation to promote maturation and differentiation into antibodies.

"We are doing studies that test the pros and cons, the yin and yang of vitamin A," says Ross. "Vitamin A has the potential to change gene expression, but that might not always be beneficial. . . . Our main question is, 'Is vitamin A effective for increasing the immune response in general, or do we need to understand its response to specific antigens?'" Ross believes that this question needs to be tested in cell and animal models "in order to know when and how to administer vitamin A wisely."

Vitamin A and Vaccines

Ross's current research focus on improving the immune response with vitamin A can be seen through a nutritional lens. "Good nutrition is such a fundamental of health, and documenting how it affects different biological systems is vital," she says. Ross is particularly hopeful that her studies will inform the use of vitamin A in public health. According to the World Health Organization, 100 to 140 million children worldwide suffer from vitamin A deficiency. Recognition that vitamin A reduces child mortality triggered a major public health effort to improve its availability in developing countries, and today many people receive a large dose of vitamin A every 4 to 6 months. But, says Ross, there has been little follow-up to determine whether the distribution of vitamin A with vaccination impacts the children's immune response.

"We now understand retinoic acid can enhance antibody production, and we hope that it can be used in a vaccine strategy for individuals in an immune-compromised state," says Ross. "That's a more pharmacological or vaccine-oriented application, but one that has potential to improve immune function." She points out, however, that anything that stimulates antibodies also has the potential to heighten an autoimmune reaction: "It's a delicate balance."

Pharmacological use of vitamin A and retinoic acid is a vast field. For example, retinoic acid is used in leukemia therapies and acne treatments. Because of retinoic acid's ability to cause tissue differentiation, the next logical step, says Ross, is to test whether low levels of retinoic acid affect differentiation of immune system cells in a way that improves immune response. Toward that end, Ross recently published findings showing that the combination of retinoic acid with the immune stimulant poly(I:C) can increase antibody responses (10). Although currently no clinical trials are planned, Ross says that such a trial is feasible, and she is close to deciding whether her laboratory will pursue this line of study.

"Many people are interested in improving vaccine efficiency, it's a huge field, but what we are saying is that nutrients should be considered in that mix," says Ross. "It would be a 'hope and dream' to improve neonatal immunity."

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