PROFILE

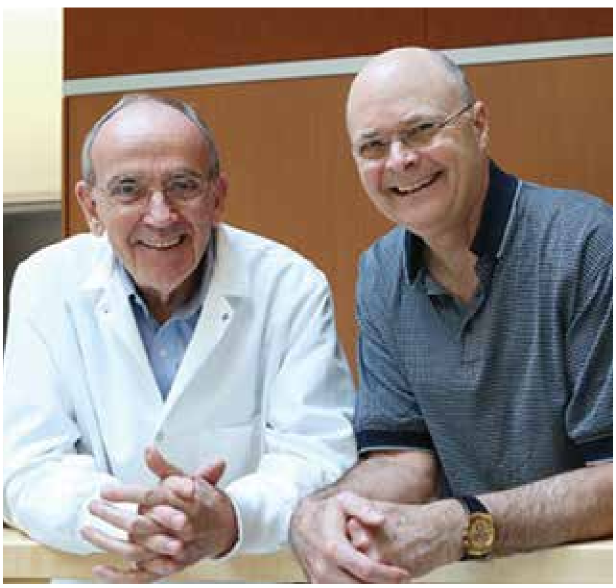
RICHARD WOLFENDEN '52 AND CHARLES CARTER '63

Of Urzymes and Enzymes

By Sarah Zobel

In response to Charles Darwin's publication of *On the Origin of Species,* one of his peers, William Thomson, 1st Baron Kelvin, a Scottish physicist and engineer, expressed skepticism. How was it possible, Baron Kelvin wanted to know, for life on Earth to have evolved so profoundly in such a short period of time?

"It sounded like a reasonable question, certainly, and Darwin had no answer to it," says Richard Wolfenden '52. "Was there enough time for all this to happen?" But Kelvin was operating on the assumption that the Earth was only about 30 million years old. "In fact, he was off by a factor of about 150," says Wolfenden, Alumni Distinguished Professor of Chemistry, Biochemistry and Biophysics at the University of North Carolina at Chapel Hill and a member of the National Academy of Sciences. In a sense, Wolfenden has been working on his own groundbreaking ideas about the origins of life, based on his research on enzymes and their role in speeding up biochemical reactions. Together with Charles Carter Jr. '63, professor of biochemistry and biophysics at UNC-Chapel Hill, he recently published two articles on the subject in the prestigious *Proceedings of the National Academy of Sciences of the United States of America*.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UNC-Chapel Hill researchers Richard Wolfenden '52 and Charles Carter Jr. '63.

The papers upended the notion of the "RNA world," a hypothesis popular among molecular biologists that assumes RNA led to the creation of proteins and, therefore, the genetic code. But Wolfenden and Carter's research shows that RNA and proteins probably evolved together. Their work is not for the scientifically faint of heart, but both men are happy to patiently explain the significance not only of enzymes, but also of molecular family trees, global temperatures, urzymes and salad dressing.

If memories of lessons learned in Exeter science classes are a bit hazy, a primer: To function, the human body requires 20 amino acids, nine of which come from food; the remaining 11 are created by the body itself. Amino acids are the building blocks of proteins, which in turn make up some 20 percent of our bodies. They're found in every cell and are at the heart of organ function, structure and regulation. In addition, the human body contains several thousand enzymes, complex proteins that act as catalysts in synchronizing chemical changes. Wolfenden has devoted years of study to the question of the degree to which enzymes speed up reactions, and, more recently, to what would happen in their absence.

"The reactions that go on when things are attached to enzymes … take place in a fraction of a second. When you don't have the enzyme there, things are much slower, but nobody had really thought to ask how much slower," he says. His idea was to increase temperature in order to measure the rates of slow reactions. Elevating the temperature from 25 to 100 degrees Celsius accelerates some reactions 10-millionfold.

In essence by increasing temperature, Wolfenden was speeding up time. After a decade or more of experimentation, he began to see a connection to Kelvin's question of evolution, and an answer: "In effect, temperature collapses time. There was plenty of time" for evolution to occur as Darwin had posited, Wolfenden says. The findings also suggested that the Earth's temperature had once been higher. By creating enzymes in the lab with amino acid sequences of their last common ancestors and then determining their properties, other investigators have found that, in fact, these so-called 'ancestral reconstructions' are enormously stable at high temperatures. "It's as if you're holding in your hand the skull of Yorick. It just jumps out at you: These ancestors lived in much hotter environments," he says.

Carter, a structural biologist, began his career with a focus on X-ray crystallography — the use of X-rays to generate high-resolution images of molecules. He has made a cottage industry of constructing family trees for molecules, which allows him to compare hundreds of different protein molecules through three-dimensional images and coordinate sets. Carter's interest in molecular evolution has focused in particular on the enzymes that translate the genetic code, which they accomplish by attaching the correct amino acids to the correct transfer RNA, or tRNA, an essential component in the creation of new proteins.

"Genes are very much like computer programs, and they're written according to a programming language that encodes the properties of the amino acids," Carter says. Amino acids, meanwhile, he likens to salad dressing, which, on standing, will separate, with some of the seasonings staying in the aqueous, or water-based, phase and the rest staying in the oil phase. Amino acids undergo the same process. Thus, an amino acid's polarity determines whether it is drawn to the oil or to the water.

Wolfenden had already been looking at the separation of amino acids between water and oil to see whether their distribution changes at higher temperatures. Together, he and Carter showed that the 20 amino acids differ not only in polarity, but also in their size or volume. Because those two measurements don't correlate, Carter believed they might form a coordinate system for defining where an amino acid will end up when a protein made of those amino acids folds. He and Wolfenden then confirmed that guess.

"Once the string of amino acids is synthesized on the ribosome, it essentially knows how to fold up like a piece of cooked spaghetti to a very compact form," Carter says. "They always fold up in a way that brings the active components into the precise locations necessary to carry out chemistry." Although there can be glitches — Alzheimer's disease is one example — for the most part, the process is universally successful. The coordinate system is sufficient to determine where any one of the 20 amino acids will land on a surface protein, a concept Wolfenden and Carter shared in one of the two PNAS articles.

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Wolfenden and Carter expect to continue their collaboration. This past spring semester, together with four other UNC professors, they co-taught a graduate-level course on the origin and early evolution of life. With just six students enrolled, it was, says Carter, "the heart of Harkness, and a number of ideas emerged from both students and faculty that are certainly worth pursuing."

In May, Wolfenden submitted for publication another paper that shows that spontaneous mutation happened 4,000 times more rapidly in the Earth's early years than it does today. "Higher temperatures mean that during the early stages of the evolution of life on Earth you've been shaking the dice frantically, producing and testing new combinations just from the effect of temperature on mutation into your natural selection," he says. "Many of the chemical bonds on which genetic information depends fall apart within a matter of days in hot water. How nucleic acids managed to store genetic information is one of the big unanswered questions."

Which brings Carter to the concept of urzymes. It's a word he coined by combining the German *ur* (original, earliest) and *enzyme*, and it refers to an ancestral synthase, much smaller than its present-day descendants, that is capable of activating amino acids, recognizing the tRNA acceptor stem, and attaching the two. How, Carter, wondered, did urzymes pair tRNA with the correct amino acid, whether oily or soluble? Using multiple regression models, he determined that in fact urzymes were capable only of recognizing the acceptor stem and therefore were operating with a set of coding principles that involve only amino acid size.

"The oldest coding that nature discovered — that we can identify — is the discrimination between small amino acids and big amino acids," Carter says. "It's a very exciting time to be interested in the origin of life because, for the first time, things that make sense are starting to emerge."

Darwin would be pleased. E