Expanding the Genetic Alphabet

New symbols added to genetic molecules form unknown messages

By IVAN AMATO

Earth's ongoing biological drama — probably the longest-running and most highly acclaimed performance in our solar system — began in a potent brew of chemicals, some of which eventually arranged themselves into the first rough drafts of life.

That's about as definite as the opening scenes of biological evolution get, constrained as they are by the limitations of current scientific knowledge. The story becomes less sketchy once the biochemistry of ancient organisms starts looking more like it does today. That turning point occurred between 3 billion and 4 billion years ago, when evolutionary caprice apparently remodeled the chemical foundations of the earliest life forms by instituting a DNA-based genetic system. Since then, every living thing that has walked, flown, budded, swam, slithered or slunk on Earth has had DNA at its biological core. (A few arguable exceptions exist in the world of viruses.) With a molecular architecture that seems miraculous in its merging of simplicity and power, DNA — the stuff of genes — continues to assemble and transform the globe's magnificently diverse biological mosaic.

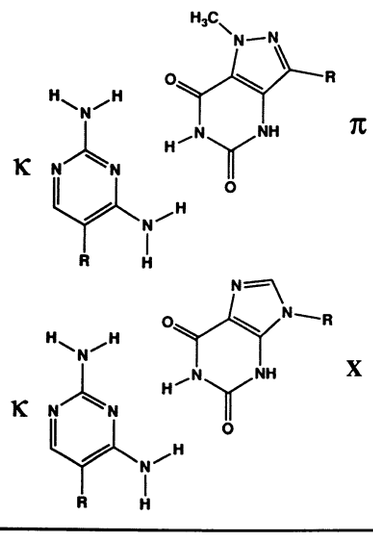
For billions of years, this chain-like molecule has had but four molecular "letters" with which to spell out the genes for such traits as long tails, brown eyes, striped fur and orange beaks. Now, scientists in Switzerland have made new letters that link up into DNA and the closely related RNA almost as if they were part of the original alphabet. Though this feat is a significant accomplishment in itself, the researchers say they hope the added symbols will someday find practical use in medicine, research and industry, carrying new chemical messages that code for a world of new catalysts and other molecular tools.

Living cells — and now scientists themselves — assemble DNA and RNA molecules as if threading beads on a string, linking chemical components called nucleotides into linear arrangements with varying nucleotide sequences (see sidebar). All the DNA and RNA molecules that have formed over the last several billion years, and therefore all the genes that have ever served as blueprints for cells, have been strung from a spartan collection of four types of nucleotides, each distinguishable by its chemical core, or base. In DNA, those bases are guanine, adenine, cytosine and thymine. In RNA, uracil replaces the thymine.

Just why DNA and RNA use only four bases remains one of the grand mysteries of biology. It was this question, coupled with the tantalizing biotechnological possibilities of making DNA and RNA from a larger set of ingredients, that sparked researchers at the Swiss Federal Institute of Technology (ETH) in Zurich to design additional bases. The enzymes that naturally assemble DNA and RNA treat the new bases virtually the same as *bona fide* members of the traditional nucleotide club, the group reports in the Jan. 4 NATURE.

"We have expanded the genetic alphabet from four to six letters," asserts molecular biologist Steven A. Benner, who leads the research team.

This laboratory achievement demonstrates that if the evolutionary drama had unfolded differently, it theoretically could have included more than four building blocks for DNA and RNA. Still, the fact that four bases have sufficed for eons of biological evolution leads many life scientists to suspect that fundamental chemical constraints prohibited additional bases from joining the molecular chains of life. "It seems we need a more subtle explanation for the four-letter code," Benner says.



Benner and his co-workers designed two new bases whose pattern of hydrogen bonds differs from the standard ones, thereby enforcing a new pattern of exclusive pairing between them. Together they represent a third Watson-Crick base pair. The new bases, kappa (κ) and pi (π), also differ from the standard bases in that they bond to the sugar component of their respective nucleotides through a carbon atom rather than through a nitrogen atom. Also shown is the naturally occurring base xanthosine (X), which shares the kappa-pi hydrogen bond pattern but lacks a biochemically "foreign" feature that might prevent polymerases from working with pi. The researchers showed that polymerases install xanthosine-containing nucleotides opposite the kappa nucleotides, and vice versa.

Why, then, would anyone bother to try to expand the genetic alphabet?

Just as adding letters to any alphabet multiplies the number of words the alphabet can form, an expanded genetic alphabet should enable researchers to build a more diverse population of DNA and RNA molecules, Benner says. Some of these RNA molecules may possess useful catalytic powers. One goal of Benner's group and a few other research teams around the world is to make a molecule that copies itself without assistance from other molecules (SN: 6/17/89, p.372). Benner says he suspects this would prove easier if scientists had a larger pool of letters to draw upon.

Gerald Joyce of the Research Institute of Scripps Clinic in La Jolla, Calif., notes that pharmaceutical companies want to develop RNA and DNA impostors, or analogs, that would counter the often-deadly activity of malfunctioning DNA or genetic material from dangerous viruses such as the AIDS-causing HIV.

And with a far more speculative tone, the ETH researchers suggest in their report that "the extra letters in the [genetic] alphabet might eventually be used to expand the genetic code." In principle, this could enable biotechnologists to design an enormously enlarged repertoire of proteins from the expanded set of amino acids that the new alphabet could potentially encode. Researchers at the University of California, Berkeley, already have developed laboratory methods for incorporating artificial amino acids into proteins. And since nature's own staggeringly diverse universe of

Nucleic acids: The threads of life

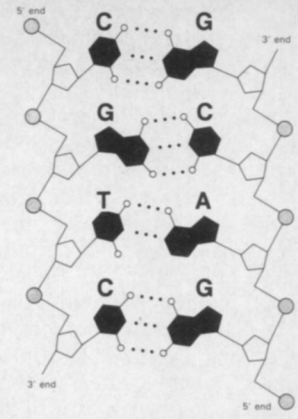
Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are composed of nucleotides, which themselves are built of three chemical components:

- bases, which carry the genetic code
- phosphoric acid groups, which link one nucleotide to another like vertebrae in a spine
- sugars (ribose in RNA, deoxyribose in DNA), which couple each base to a phosphate group.

The bases fit into two chemically complementary categories – the purines and pyrimidines. The term purine denotes a molecular framework marked by a five-sided ring of atoms fused to a six-sided ring. Adenine and guanine serve as the purines in both RNA and DNA. In DNA, cytosine and thymine are the pyrimidines, characterized by their single six-sided ring. In RNA, uracil replaces the thymine.

DNA and RNA chains form when enzymes called polymerases sequentially link adjacent nucleotides. In RNA and in single-stranded DNA molecules, purines and pyrimidines can link in virtually any sequence of virtually any length. But doubled-stranded DNA can form only when the sequences of bases on the two strands are complementary. And this occurs only when the adenines of one strand line up with the adjacent strand's thymines and all of the guanines line up with the cytosines. The adenine-thymine and guanine-cytosine pairings are known as Watson-Crick base pairs. The pattern of hydrogen bonds in each of the two pairs enforces exclusive pairings.

With the help of polymerases, double-stranded DNA can make multiple copies of itself because each of its two strands serves as a chemical "negative" for the other. When the strands separate, one acts as a template upon which a DNA polymerase can assemble a new, complementary strand. The result: a pair of identical, double-stranded DNA molecules.



Patterns of base pairs in DNA

To transcribe DNA into complementary RNA, an RNA polymerase uses one strand of the DNA duplex as a template for assembling a complementary sequence containing uracils wherever the DNA sequence had an adenine base.

– I. Amato

Figure from *Molecular Cell Biology*, Darnell et al. © Sci. Am. Bks., w/permission, W.H. Freeman

proteins – which function as both structural molecules and enzymes – springs from only 20 types of amino acids, additional amino acid ingredients could expand that universe into unfathomable dimensions.

But first things first. Benner readily admits that creating a new universe of proteins from an expanded genetic code remains wishful thinking for the moment. So far, he and his colleagues have gotten RNA and DNA polymerases – cell-derived enzymes that link nucleotides into RNA or DNA chains – to reliably install a synthetic genetic "bead" into short chains of DNA and RNA. While a number of researchers have achieved somewhat similar feats in the lab using clever chemical techniques that do not involve enzymes, the new work marks the first time scientists have coaxed cellular enzymes into threading nonstandard nucleotides along the same string as the traditional ones.

When DNA in a cell nucleus becomes active, it either replicates (during periods of growth or reproduction) or serves as a template for making complementary RNA molecules (during normal cell metabolism). This RNA then carries the genetic code out of the nucleus to other cellular sites, where ribosomes read the so-called messenger RNA like data tape to guide the assembly of proteins. The fidelity of DNA replication or its transcription into messenger RNA results from the exclusive molecular relationships between certain pairs of bases on the genetic beads.

The bases fall into two complementary chemical categories: purines, which have two fused rings of atoms; and pyrimidines, which have a single ring structure. In DNA, the purine base adenine always pairs with the pyrimidine base thymine, and the purine guanine always couples with the pyrimidine cytosine. These two combinations are known as the Watson-Crick base pairs. Other combinations of the bases do not last long because they lack the patterns of stabilizing hydrogen bonds that strongly favor the formation of the Watson-Crick base pairs.

Benner and co-workers Joseph A. Piccirilli, Tilman Krauch and Simon E. Moroney set out to design a third Watson-Crick base pair. The synthetic couple would have to be so similar to the traditional base pairs in shape, size and chemistry that the RNA and DNA polymerases would recognize them as eligible genetic beads. Yet any new Watson-Crick base pair would also require its own exclusive pattern of hydrogen bonding to prevent unwanted pairings between a new base and a standard base. (Earlier Watson-Crick-pair candidates allowed too many mispairings to be useful.) Without this inviolable one-to-one pairing, DNA replication or transcription into messenger RNA would lack the precision needed for reliable reproduction, growth or protein manufacturing. In short, life in its present form would be impossible.

In their NATURE article, the researchers report designing and making a new Watson-Crick base pair that almost meets these criteria. The new pyrimidine, which they named kappa, differs from the traditional pyrimidines (cytosine, uracil and thymine) in the orientation of its nitrogen atoms. As a result, the pattern of hydrogen bonds it can form with its new purine – designated pi and designed for exclusive complementary-pairing with kappa – is unique.

When the scientists chemically synthesized pairs of complementary strands of DNA, including the new kappa-pi Watson-Crick pair, they were pleased to find that the strands bonded to each other with nearly as much stability as normal strands lacking the "funny bases," as Benner calls them.

"These results indicated that enzymatic incorporation of a new base selectively opposite its complement in a DNA template would be possible," the team concludes.

But pi's molecular structure has a small chemical side group that the natural purines lack. Although the researchers found this difference unimportant when they used strictly chemical means for linking nucleotides, they feared it would prove too exotic for recognition by the RNA and DNA polymerases. So, for their enzymatic studies, they chose to use xanthosine, a naturally occurring purine with a structure very similar to pi. Xanthosine retains the exclusive hydrogen-bonding pattern of the kappa-pi pair but lacks pi's foreign-looking chemical group.

The kappa-xanthosine pair performed as the researchers had hoped. When they made short DNA template strands that included a kappa bead, the RNA and DNA polymerases assembled complementary strands with the xanthosine bases always opposite the kappa. Errors were nearly as rare as in control experiments using traditional base pairs, the researchers note in their report. And in the time since

the paper was written, ETH's Christopher Y. Switzer has shown that installing xanthosine in the template directs the polymerases to faithfully incorporate kappa into the appropriate spots on product strands, Benner told SCIENCE NEWS.

"The real goal here is to have an RNA molecule that's able to replicate itself," says Benner. That would be a first step toward such visionary goals as creating lifelike chemical systems in a test tube. Finding self-making molecules, which would catalyze their own copying without the help of specialized enzymes like polymerases, is the golden ring for a small community of origin-of-life researchers.

There may be some bugs with the new letters, cautions Leslie Orgel of the Salk Institute for Biological Studies in San Diego, in a commentary accompanying the research report. For one, he notes, the xanthosine base carries an extra bit of negative charge that could prevent the forming of double helices or other molecular arrangements important for biological activity.

Says Benner: "With one funny base in a backbone of normal bases, we don't see any problem." But he concedes that no one knows how longer chains with more funny bases will behave. "We have taken a step in a rather long series of steps that is necessary for getting something that is self-replicating."

Orgel questions the need to add letters to the genetic alphabet for the goal of building new catalytic RNA molecules. "Maybe four are enough [to work with]," he suggests. In addition, he says, chemists are getting so good at chemically synthesizing RNA that the use of harder-to-handle enzymes could soon become more trouble than it's worth.

But Benner suspects otherwise. By adding bases with different chemical features to the pool of ingredients for making RNA molecules, he says, researchers will have more to work with in designing catalysts for performing specific chemical transformations. Even with the Berkeley group's advances in making proteins with synthetic amino acids, deliberate engineering of catalytic RNA to perform specific molecular jobs may still emerge as an easier task, Benner asserts. Protein designers still do not understand how linear sequences of amino acids fold into the three-dimensional arrangements they must assume to function properly. "In RNA, we actually understand how this works," Benner says. Combining this understanding of structure with a larger variety of nucleotide building blocks will better enable researchers to understand and control RNA molecular structure and, therefore, to design RNA-based catalysts, he predicts.

Manipulating bases isn't the only way in which scientists have tweaked the structures of RNA and DNA. "People have tried to change everything," says Joyce of the Scripps Clinic. For instance, he notes, another ETH scientist has replaced the nucleotides' five-membered ribose sugar component with the six-member glucose. "Basically any sugar has been tried," says Joyce.

Stanley Miller of the University of California, San Diego, experiments with bases that have more open structures, lacking the rings characteristic of the bases in normal DNA and RNA. Others have tried changing the type of chemical bond that links the nucleotides. Benner, for instance, is building DNA- and RNA-style structures with sulfur-containing chemical bridges instead of the normal phosphorus-oxygen-phosphorus link. Orgel has tried using an amide linkage, the same type of chemical bond that links amino acids into proteins.

Driving many of these experiments is the scientific enigma of how life began. After scrutinizing the molecular biology of living organisms, many origin-of-life researchers have come to suspect that a so-called RNA world preceded the DNA-based life forms that have presided for more than 3 billion years (SN: 10/7/89,

Continued on p.94

News of the week continued from p.87

Thomas Dickinson says the experiments show unambiguously that a crucial condition for fracto-fusion — charge separation across microcracks in a solid — does occur. In addition, he notes, calculations reported by Japanese researchers suggest that a growing crack, even in an electrically conductive material, can outpace separated charges attempting to neutralize each other by speeding around the increasing perimeter of a crack.

In principle, the oppositely charged sides of a microcrack could create a strong electric field that would greatly accelerate positively charged particles, such as deuterium nuclei, in the gap. This would increase the probability of deuterium-deuterium fusion reactions, Dickinson's group suggests.

As early as 1986, Soviet scientists reported observing neutron emission when they violently crushed lithium deuteride in the presence of an ice made of heavy (deuterium-containing) water. In a letter published in the Nov. 16 NATURE, they describe more recent experiments involving titanium chips and several deuterium-containing materials, including frozen heavy water and lithium deuteride. While vigorously milling the titanium and the deuterium sources, and for a few minutes after milling had ceased, the Soviet researchers detected neutrons emerging at up to seven times the levels measured for titanium chips or deuterium sources milled separately. They suggest that fracturing may play a role.

Dickinson's group placed successive slabs of hydrogen-loaded titanium, deuterium-loaded titanium and unloaded titanium in an apparatus that bends materials until they crack and finally break. During and shortly after fracture, the researchers recorded each specimen's emissions of positively charged particles and photons of various wavelengths. Though they had expected the two gas-loaded materials to yield similar results, they found that the deuterium-loaded specimens produced far stronger signals. "The differences in the fracto-emission between these two types of specimens were astounding," they report.

The identities and energies of the emitted particles and radiation remain unknown, and Dickinson wants to conduct follow-up experiments to answer those questions. He notes, however, that funding for cold fusion research has become scarce, especially since last November, when the Energy Department issued a report essentially writing off cold fusion claims as unfounded (SN: 7/28/89, p.78).

Like many physical scientists, Harold Furth, head of the Plasma Physics Laboratory at Princeton (N.J.) University, trains a critical eye on any proposed mechanism for cold fusion. Although Furth himself casually mentioned the possibility of fracto-fusion last May at a meeting of the American Physical Society, he says he now suspects the entire cold fusion drama sprang from misinterpretations of data and experimental errors. "I wouldn't rule out that this [fracto-fusion] is zilch," he told SCIENCE NEWS.

In the midst of such skepticism, physicist Steven E. Jones of Brigham Young University in Provo, Utah, maintains that "fracto-fusion probably is the leading model right now." Jones headed one of the two independent research teams that initially announced the possibility of achieving cold fusion by using electrochemical processes to jam deuterium into metal rods (SN: 4/8/89, p.212). Although he admits that the evidence for fracto-fusion remains inconclusive, he and collaborators at Los Alamos are assembling a sophisticated apparatus that may help settle the issue. By injecting tritium into gas-loaded titanium samples and using hydraulic presses to squeeze and fracture the specimens, the team hopes to increase the probability of fusion by several orders of magnitude, Jones told SCIENCE NEWS. If any fusion-produced neutrons do emerge, the apparatus should allow researchers to detect them and measure their energy, he says.

— I. Amato

Continued from p.90

p.229). They argue that the ancient RNA, while performing many of the enzymatic functions now done by proteins, also served for a time as the sole carrier of genetic information.

But RNA itself may have evolved from an even earlier information carrier. When scientists reconstruct what they envision as the most likely conditions of the prebiotic chemical brew, they find no reason for the present form of RNA to have predominated over other, equally plausible molecular structures. Rather, they speculate that something else – something that could copy itself and also evolve into the present forms of RNA – embodied the very beginnings of life. Experiments like those of Benner, Orgel and Miller help constrain such theories. Virtually all scientists in this field hope that one day they may find life in their test-tube versions of the primordial womb.

"This is different from usual science," Miller remarks. "You're trying to reconstruct an historical event – the origin of life." And good things happen along the way. "As one tries to go through the steps of constructing these self-replicating systems, you encounter all sorts of chemistry that you would not encounter otherwise," Benner says. "It's a problem not many people look at, and it's one that is terribly central to understanding anything about living systems." □

The Care and Feeding of Ideas: A Guide to Encouraging Creativity

By James L. Adams

Good ideas are not easy to cultivate. They need rich soil, attention, and encouragement to take root and grow. This book is a guide to creating a greenhouse environment in which ideas can thrive. Adams leads a tour through the unconscious mind, the brain and nervous system, and the storehouse of memory, and points out how they work (and conspire against us) when we tackle problems. He shows that bad problem-solving habits can be broken, that money and time are your muse's best friends, that creativity involves risk but the risk is worth it, and that the stereo you bought with your last bonus was not a luxury but a necessary reward. He illustrates his arguments with ingenious games and exercises that will surprise you with what they reveal about your patterns of thought.

— from the publisher

Care & Feeding, Addison-Wesley, 1986, 223 pages, 9¼" x 6¼", paperback, $9.95. **Blockbusting,** 1974, 161 pages, 9¼" x 6¼", paperback, $9.95.

Conceptual Blockbusting: A Guide to Better Ideas

By James L. Adams

In one way or another, everyone needs to be creative. Yet all of us are beset by psychological barriers that prevent us from finding the creative solutions to our problems. Adams shows readers how to recognize each of these blocks – from the emotional to the cultural – and, more important, he offers techniques for busting through them. *— from the publisher*

"This book has a way of helping you stand problems on their heads so you can solve them."

Donald Kennedy, President, Stanford University

Science News Books, 1719 N Street, NW, Washington, DC 20036

Please send the books marked below.

_____ **The Care & Feeding of Ideas** $9.95
_____ **Conceptual Blockbusting** $9.95

Please include $2.00 postage and handling for each item ordered. Maximum $4.00 charge.

Name _____

Address _____

City _____

State _____ Zip _____

RB1186