

Celebrating Complementarity

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The 1950s witnessed prodigious growth in our knowledge of the chemical nature of the gene, the mechanisms by which the information inherent in its sequence of nucleotide units was conveyed to the cell's machinery for synthesizing proteins, and the mode of operation of that machinery. Watson and Crick raised the curtain by revealing the structure of DNA, notably the complementary pairing of the nucleotides in its twin chains. Identified with the new field of molecular biology, Crick defined and expanded the field of genetic information coding and led attempts to elucidate principles governing information translation into protein. On a parallel and complementary track, biochemists—notably the group led by Paul Zamecnik—were working out the details of the mechanism of protein synthesis: how and where in the cell amino acids were energized, sequenced, and polymerized. These two approaches to closely related problems, separated by a considerable

cultural gap, merged dramatically in 1956. Crick's thinking led him to postulate the existence of *adaptors*—short chains of RNA nucleotide bases that, when linked to amino acids, might make the latter chemically “recognizable” to an RNA template by complementary pairing of their bases with those of the template. Simultaneously, the Zamecnik group discovered enzymes (amino acyl-tRNA synthetases) capable of energizing amino acids and, thence, attaching them to a hitherto unsuspected cellular RNA (transfer RNA). These RNA molecules, in turn, donated their bound amino acids to elongating protein chains on ribosomes, thus appearing to serve the function of adaptors.

Ann Intern Med. 2003;138:583-586.

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For expanded accounts of the story related here, see the Appendix.

In April 1953, on a colleague's desk I saw a copy of *Nature* open to the paper of Watson and Crick. I gave it a cursory read and thought, “Interesting—not down my alley.” That my reaction to a discovery of such importance was so naive was due, I suppose, to the newness of my entry into biology and to science's compartmentalization. I was 5 years out of medical school, immersing myself in biochemistry in a medical milieu hoping to solve some mysteries of cell growth and cancer, far removed from any interest in the physicochemical properties of large molecules.

In the years between the mid-1940s and the late 1950s, biological science was intensely focused on two central problems: 1) the nature of the gene and the processing of genetic information and 2) the mechanism of protein synthesis. The former was the realm of those who came to be identified as molecular biologists. The latter attracted the classical biochemists, who liked digging into the energetics, enzymology, and compartmentalization of cellular machinery. Although these two approaches were separated by a wide cultural gap, they were complementary to one another and were destined to merge, unexpectedly and dramatically, in 1956. That year, biochemists found transfer RNA to be the link between the gene and the assembly of protein, and molecular biologists independently forecast an explanation for its existence.

The rediscovery of Mendel's laws of inheritance at the beginning of the 20th century and the ensuing burgeoning of research in genetics had, by the mid-1940s, brought science to a critical issue: the physicochemical nature of the gene. In 1943, the physicist Erwin Schrödinger framed the question that came to preoccupy many scientists: “How can we reconcile the facts that the gene structure seems to involve only a comparatively small number of atoms . . . and that nevertheless displays a most regular and lawful activity—with a durability or permanence that borders on

the miraculous.” A great leap forward was, of course, the nailing of the right molecule: the experimental demonstration by Avery, McLeod, and McCarty in 1944 and by Hershey and Chase in 1952 that DNA was, in fact, the material of the gene. Their findings were proximal causes of Watson and Crick's plunge into the structure of DNA. The model that Watson and Crick produced in 1953 arose from an astute appraisal of DNA's chemical properties and a critical interpretation of its x-ray diffraction patterns. It immediately and dramatically offered a satisfying explanation of DNA's durability and the mode and precision of its replication—centered on the double molecule's complementary pairing of its nucleotide units. But of what did DNA's *information* consist? The only possibility seemed to be the incredibly long molecule's sequence of nucleotides (its four code letters: A, T, G, and C)—the equivalent, perhaps, of a written set of instructions.

During those same germinal years, the biochemists focused on the corollary question: How was this information, whatever form it took, conveyed to its vehicle, the living cell? The team headed by Paul Zamecnik in Harvard's Department of Medicine at Massachusetts General Hospital, which I would join, saw fractionation and reassembly of the machinery of protein synthesis as the route to an answer. The team sought ways to break open cells so as to preserve their vital functions, isolate key components, and put them back together so they'd carry out protein synthesis in the test tube. (The source of the cells for most of their work was the rat liver.)

Back in the early 1940s, biochemists in Sweden and Belgium made the first observations correlating a high content of cellular RNA (ribonucleic acid) with rapid rates of cell growth and of protein synthesis. And the electron microscope was beginning to reveal that the cytoplasm of both animal and bacterial cells was loaded with tiny dense spherical particles, which came to be called ribosomes be-

cause they contained most of the cells' RNA. In bacteria the ribosomes seemed to be free, while in animal cells they were largely attached to intracellular membranes—the whole constituting the endoplasmic reticulum. When liver cells were broken open and their contents separated in the ultracentrifuge—first removing the larger nuclei and mitochondria—the endoplasmic reticulum sedimented as material called microsomes. Zamecnik and his group had shown that when rats were injected with amino acids labeled with the radioisotope of carbon— ^{14}C —the microsomes isolated from their livers were the first to incorporate the label into protein. (The group pioneered the use of ^{14}C -labeled amino acids in the study of protein synthesis, an enormously valuable assay.)

Subsequently, the refinement of a cell-free system for synthesizing protein from rat liver, largely in the Massachusetts General Hospital laboratories, moved forward in the early 1950s. As expected from the *in vivo* studies, microsomes were again the initial site of appearance of new protein. And now a soluble cell fraction—material that remained in solution after the microsomes had sedimented in the ultracentrifuge—proved to be another essential cellular component. In addition, both adenosine triphosphate (ATP) and guanosine triphosphate were required for protein synthesis in this cell-free system.

I joined the group in 1953, after having spent a year in the laboratory of Fritz Lipmann, also at Massachusetts General Hospital. Lipmann was a pioneer in biochemical energetics and had a strong influence on those of us concerned with how ATP might participate in the linking of amino acids. Using techniques of radioactive phosphate exchange that I'd acquired in Lipmann's laboratory, I discovered, in 1954, the initial step in protein synthesis—the activation of amino acids, the process by which the energy of ATP is donated to amino acids—catalyzed by enzymes residing in the soluble fraction:

$$\text{ATP} + \text{amino acid} \rightarrow \text{AMP-amino acid} + \text{pyrophosphate}$$

The carboxyl group of the amino acid becomes linked to the phosphate of adenosine monophosphate (AMP), releasing the two terminal phosphates of ATP as pyrophosphate. Preliminary evidence indicated that each of the 20 amino acids was activated by a separate enzyme and that the intermediate AMP-amino acids were tightly bound to their respective activating enzymes. (It has always puzzled me why Lipmann had not already tackled the problem of amino acid activation. It had long intrigued him, and he and his associates were then working on the role of ATP in energizing other biochemical processes.)

The discovery of this first step in protein synthesis delighted the classical biochemical community. The molecular biologists, on the other hand, were not moved. I first reported the discovery at a meeting of molecular biologists in 1955, and the palpable indifference with which the audience received the news showed how firmly closed was the door between the two groups. The focus of the meeting

was on how units were *ordered*—the realm of the molecular biologists—not on how they were *energized* and mechanically polymerized—the realm of the biochemists.

Inspired by the Watson-Crick model, the physicist George Gamow was the first to suggest some specific ideas about how arrangements of nucleotides in DNA might specify the sequence of amino acids in protein. He communicated his scheme (ingenious but erroneous) to Crick in the summer of 1953, and Crick thenceforth directed much of his attention to what he came to call “the coding problem” and “the sequence hypothesis.”

It was widely accepted by this time that an RNA template—a sequence of nucleotides specifying an amino acid sequence, copied from DNA—must exist in the ribosome. The coding ideas that had thus far been generated envisioned some direct interaction between amino acids and groups of nucleotides in an RNA template. They ran head on into the daunting fact that there was simply no chemical similarity, or complementarity, between the two sets of molecules. Crick, in an imaginative leap out of that trap, postulated an intermediary: something that might combine with an amino acid to make it recognizable to a nucleotide, or to a short sequence of nucleotides, in an RNA template. The obvious candidate, at first scrutiny, was an enzyme—in fact, 20 different enzymes, one for each amino acid. Enzymes had the necessary specificity to recognize both amino acids and, possibly, a particular sequence of nucleotide in a chain. At this time (1954), Crick's colleague and fellow thinker about coding, Sydney Brenner, suggested a name for such an intermediate molecule: *adaptor*, a molecule that would make an amino acid recognizable—would adapt it—to a template.

As Crick's thinking evolved, influenced by his awareness that RNA was somehow an obligatory participant in protein synthesis and that complementary pairing of nucleotides in RNA, as in DNA, was a source of specificity, he shifted emphasis from protein to RNA as adaptor. The template, Crick hypothesized, might most readily and specifically interact, by nucleotide pairing, with an amino acid-bearing adaptor if that adaptor were itself a complementary stretch of nucleotides. In its simplest form, 20 specific enzymes would catalyze the attachment of 20 different amino acids to 20 different RNA adaptors, and these would then be ordered by nucleotide pairing on an RNA template in the ribosome.

In early 1955, Crick wrote up this stunning insight in a paper called “On Degenerate Templates and the Adaptor Hypothesis—A Note to the RNA Tie Club.” It was circulated among the molecular biologist members of the club (founded by Gamow a few years before, its 20 members each named for one of the 20 amino acids found in protein) and was never published. (Crick did make brief note of the adaptor idea at a London symposium of the Biochemical Society, and the proceedings were subsequently published.)

Among the biochemists, RNA was also getting increas-

ing attention. Paul Zamecnik had been wondering, as a result of the discovery of amino acid activation, whether the key intermediate—AMP–amino acid—might be “double-barreled”: that is, able to participate in the synthesis of RNA by donating its AMP moiety to a growing nucleotide chain. Perhaps the activating enzymes, under certain circumstances, could catalyze such a reaction. In late 1955, Zamecnik tested the idea by incubating ^{14}C -labeled ATP with microsomes and soluble fraction, and found that the label did indeed appear in RNA. With characteristic caution, Zamecnik suspected that the labeling of the RNA might be a nonspecific association—a contamination—so he repeated the experiment using a different radioactive label, ^{14}C -labeled amino acid, expecting that it, having no chemical relationship to RNA, would not become linked to RNA. But to his surprise, it did. And, more astonishing still, the label was not in the RNA of the ribosomes but, instead, in a small fraction of RNA in the soluble fraction, 10% of the total RNA in the combined microsome–soluble-fraction system. Finally, when he incubated only the soluble fraction with labeled amino acid and ATP, the amino acid became linked to this new species of RNA. The existence of this minor cellular RNA, which we called sRNA, for soluble RNA, had earlier been noted by other workers in the field and had been set aside as “junk”—considered likely fragments of the much more plentiful ribosomal RNA produced during the traumatic process of breaking open cells.

I pursued the lead and found that amino acids were covalently linked to sRNA by separate enzymes in the soluble fraction, presumably the same enzymes responsible for the initial ATP-dependent activation step. Thus, our new formulation became:

$$\text{amino acid} + \text{ATP} \rightarrow \text{AMP–amino acid} + \text{pyrophosphate}$$
$$\text{AMP–amino acid} + \text{sRNA} \rightarrow \text{amino acid–sRNA} + \text{AMP}$$

The amino acid remains in the activated—energized—state on sRNA. On the basis of the fact that each of several amino acids became linked to sRNA separately and noncompetitively, we assumed that multiple binding sites existed either on the same sRNA or on different sRNA molecules.

In an experiment that remains as vivid in my mind as though I'd done it yesterday, I asked the key question: Would sRNA–amino acid transfer its amino acid to protein in the absence of ATP (since the amino acids are now in the activated state)? I combined sRNA charged with ^{14}C amino acids, the soluble fraction, microsomes, and guanosine triphosphate and incubated them briefly. To our delight, amino acids rapidly disappeared from sRNA and appeared in protein. From that moment on, we had little doubt that the small RNA species was the physical link between activated amino acids and their ultimate ordered arrangement in protein. It would not be long before sRNA was re-

named tRNA (transfer RNA); the amino acid–activating enzymes were renamed amino acyl-tRNA synthetases.

This was clearly a discovery whose time had come. Not only had it been anticipated on purely theoretical grounds, but, independently, in Japan, Ogata and Nohara were doing experiments very similar to ours, and three other teams, one in Sweden and two in the United States, were beginning to detect amino acid binding to RNA.

Upon learning of our discovery of tRNA's participation in protein synthesis, Jim Watson, recently settled in Harvard's Department of Biology, paid us a visit in late 1956. He told us of the adaptor hypothesis and seemed already convinced that our sRNAs were Crick's adaptors. We were taken aback and somewhat miffed that a molecular biologist had postulated the existence of—and the function of—a key intermediate in a process that we biochemists had independently discovered. But the adaptor idea was so soul-satisfyingly correct that one had to accept it with open arms. We had to acknowledge that we'd come across cellular machinery that accommodated the need for coded sequencing without giving much thought to the coding problem.

And so it was that the dual discovery of amino acyl-tRNA synthetases—and of tRNA, together with the theoretical framework of the adaptor hypothesis, breached the wall between biochemistry and molecular biology.

Crick visited us early in 1957 and was, of course, delighted with our findings. He was troubled that tRNA molecules were so unexpectedly big, some 60 to 70 nucleotides long. He'd envisioned adaptors as quite small, even as few as 3 nucleotides, no more than needed to code for 20 amino acids. He'd hoped that we might begin to ferret out a coding triplet for an amino acid by locating it on its cognate tRNA. He invited me to join him for a year (1967–1968) at Cambridge. There we shared a small laboratory and spent a few weeks attempting to fractionate sRNA into separate species—unsuccessfully. The depth of Crick's plunge into biochemistry is illuminated by a scene I remember vividly in the early days of that year in Cambridge. He habitually arrived late at work, but he'd been coming to work early then, eagerly learning how to do what biochemists do. As I entered the lab, I was surprised to find him on hands and knees under a laboratory table trying to capture a rat that was eluding his efforts to use its liver as a source of tRNA.

Our hopes of approaching the code via tRNA faded, and we spent most of the year talking science and enjoying the company of many visiting colleagues. It was a year that saw rapid progress in the experimental proof that single nucleotide alterations in a gene were collinear with single amino acid changes in a protein, changes fine enough to lead to the conclusion that three nucleotides coded for one amino acid (a coding ratio of 3). And I spent 2 weeks that winter at the Pasteur Institute in Paris with François Jacob, Jacques Monod, and Arthur Pardee just as they were un-

earthing critical evidence for the existence of messenger RNA—the long-sought template.

The complementarity of the efforts of the biochemists and the molecular biologists merged with a final flourish. A long-sought *in vitro* protein synthesizing system derived from bacteria (*Escherichia coli*) was developed by Marvin Lamborg and Paul Zamecnik in 1959. It rapidly supplanted the rat liver system in many laboratories and was soon used in Marshall Nirenberg and J. Heinrich Matthaei's dramatic cracking of the genetic code (1961).

By then it had been only 8 years since I first glimpsed the Watson and Crick paper. Looking back now over the 50 intervening years, I marvel at the brilliance of the explosive synthesis of knowledge that was launched by their drive to envision the gene in its chemical beauty.

APPENDIX: RELATED READING

Judson HF. *The Eighth Day of Creation: Makers of the Revolution in Biology*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Pr; 1996.

Rheinberger HJ. *Toward a History of Epistemic Things. Synthesizing Proteins in the Test Tube*. Stanford, CA: Stanford Univ Pr; 1997.

Hoagland M. *Toward the Habit of Truth: A Life in Science*. New York: W.W. Norton; 1990.

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Potential Financial Conflicts of Interest: None disclosed.

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