The Protein that Wasn't There: The Discovery of Ribozymes

Introduction

If we were challenged to describe, in layman's terms, what makes living matter different from non-living matter, I suspect that many of us would focus on nucleic acids. Their ability to encode information, to replicate, and their passage from one generation to the next is part and parcel of what makes life special. Ironically, if one examines an organism carefully and observes what makes it "alive," nucleic acids turn out to have very little direct effect on living matter. Whether an animal moves, breathes, digests, turns its head to look, or just blinks an eye, its actions depend far more on enzymes than they do on DNA.

Enzymes in muscle produce movement, in nerve cells they open membrane channels that produce message-carrying impulses, throughout the body enzymes produce, store, and convert chemical energy from one form to another. Enzymes are biological catalysts. Enzymes, which lower the activation energies of chemical reaction, are responsible for just about every activity that we associate with living things. Indeed, enzymes even control the synthesis, replication, folding, and activation of DNA itself.

What kinds of molecules are there marvelous enzymes? They are proteins, of course. Look at any biology text, high school or college (including pp. 74-76 of **Biology** by Miller and Levine), and you will find that they devote a generous amount of space (and lots of pictures) to the structure of proteins.

They are loaded with terms like a- helix, b- sheet, prosthetic group, secondary structure, and so forth. Following right on the heels of the protein structure section will be an extensive discussion of enzymes. It will mention enzyme-substrate complexes, active sites, inhibitors, regulators, and reaction rates. The close association between these two topics stems from the fact that enzymes are proteins. Until very recently, there were no exceptions to that rule.

In a chemical sense, this left nucleic acids as nothing more than repositories of information. Like any store of information, there could be no doubting their importance, but they certainly were dull when compared to the extraordinary chemical tricks that could be pulled off by enzymes. Indeed, I once heard a biochemist claim that the only reason that DNA was even slightly interesting was because the instructions for enzyme construction were written in DNA, and that only made the nucleic acid worth studying.

For most of the era of molecular biology the distinction that proteins held as being the only molecules capable of acting as catalysts stood unchallenged. Then something happened. A young professor's graduate students kept getting a result that didn't make sense. They first thought they were doing something wrong. It turned out that they were doing something right, and they helped to spark a revolution in biochemistry.

RNA Splicing in *Tetrahymena.* Who Cares?

In 1978, Thomas Cech, a young Assistant Professor of Chemistry at the University of Colorado, was looking around for a "system" in which he could study RNA cutting and splicing. A couple of years earlier, scientists around the world had made an exciting discovery: transcription, the production of RNA against a DNA template, was not the final step in the production of RNA. Many RNA molecules were cut and spliced before they were ready to go to work. In many messenger RNAs, large pieces were literally cut out of RNAs, thrown away, and the remaining pieces were then spliced together before the molecule was ready to go to work directing the synthesis of polypeptides.

Like many researchers, Cech realized that next to nothing ws known about how the splicing took place. He looked around for an organism in which it would be easy to isolated large amounts of RNA and study the

cutting and splicing reactions. The organism he selected was a ciliated protozoan called *Tetrahymena (Tetrahymena* is quite similar to *Paramecium,* which is described on pp. 384-386 of **Biology**). As odd as the protozoan might seem, Cech realized that *Tetrahymena* had several advantages. He could grow lots of it in pure culture in the lab, the cells grew rapidly, dividing every 2.5 hours, and each cell contained a very active macrinucleus in which several genes were present is multiple copies. Knowing that he needed an abundant source of RNA, he selected the ribosomal RNA (rRNA) gene, which is present in more than 40,000 copies, and produces an enormous amount of RNA (enough to double the cell's supply of ribosomes every 2.5 hours).

Like many researchers, he had chosen a biological system not because he was intrested in the organism itself. *Tetrahymena* doesn't cause disease and is of no economic importance. Rather, he had selected *Tetrahymena* as a "model" system because he thought it presented an excellent chance to learn about RNA splicing. He could then take the lessons his lab learned from *Tetrahymena* and apply them to other organisms. It would take at least 3 years before Cech appreciated how lucky his choice was.

Looking for a Splicing Enzyme

Cech and his students quickly discovered that Tetrahymena rRNA had an intron, an intervening sequence (see Biology pp. 219-220) that was spliced out before the rRNA was ready to go to work in forming part of a ribosome. They decided to use a classic biochemical strategy -- to separate the unspliced rRNA from everything else inthe cell, and then gradually add back bits and pieces of the cell until that found a fraction that contained the splicing enzyme.

They first developed a method for isolating nuclear fragments that were active in transcription (RNA production), and discovered to their surprise that this preparation was still active in splicing. No problem. They figured that the splicing enzyme must be so tightly bound to the RNA that it was difficult to separate. In 1979 Cech and two of his graduate students, Paula Grabowski and Arthur Zaug, published a paper in which they described the isolation of this nuclear fraction. They confidently predicted that this isolated fraction would enable them to "find the splicing enzyme" in a matter of a few months. Cech even wrote a grant proposal entitled "Enzymatic Splicing of rRNA Precursor." It was quickly funded. Everyone awaited the isolation of the splicing enzyme. They were to be disappointed.

The Experiment that Never Worked

The Colorado researchers tried every trick they knew to remove enzymes from the nuclear extract. Despite their best efforts to remove proteins from the mixture, splicing still took place. The intron was cut out, thrown away, and the two ends of the rRNA were spliced back together. Tom Cech said: "I said, 'This can't be right.' Art Zaug repeated it five times, but each time with the same result. Even then we didn't believe it, so we put it aside."

Since they couldn't find the enzyme, they spent nearly a year and a half investigating the chemical events of the splicing reaction itself. What they discovered was that the rRNA bent back upon itself forming a loop that contained the entire intervening sequence. That loop was then broken away from the rest of the molecule and discarded. The parts on either side of the loop then joined together to form the final rRNA molecule. Little by little it because clear that this could happen without an enzyme. In a sense, the rRNA molecule *itself* was the enzyme.

One at a time the investigators convinced themselves that they were right. The RNA molecule was catalyzing its own splicing! In 1982 they finally wrote a paper reporting the result, and descibing all of the experiments they had done to show that this rRNA was indeed a self-splicing molecule.

Implications

At first, one might be tempted to conclude that *Tetrahymena* was just another one of those interesting exceptions that biology is full of. However, their 1982 paper was written with full knowledge that it contained something revolutionary: the first-ever description of a non-protein enzyme. RNA could be an enzyme, too!

A few purists pooh-poohed this conclusion, pointing out that true enzymes catalyze reactions involving *other* molecules, not themselves. So the self-splicing RNA might not be such a big deal after all. However, other researchers were stimulated by the Colorado paper to look for RNA in other chemical reactions, and within a year there was a spectacular confirmation of their general conclusions.

Sidney Altman of Yale, working with Norman Pace in Denver, had cooperated for several years in studying the activities of ribonuclease P, an enzyme involved in the processing of tRNA molecules. This enzyme had always been a curiosity, since it contains its own RNA. In fact, about 80% of the mass of the enzyme is RNA, and only 20% protein. Most other biochemists, however, thought that the RNA in ribonuclease P was either unimportant or a leftover from poor isolation techniques.

Students in both laboratories then showed that the reaction catalyzed by ribonuclease P could be carried out, under the right conditions, by its RNA alone. In short, that the catalytic part of the enzyme was RNA, not protein. RNA really could catalyze a chemical reaction. In fact, RNA was the most important component of ribonuclease P.

The Ribozyme

A flood of discoveries followed this groundbreaking work, and even a few new terms. First, the generalization that all enzymes are proteins is clearly wrong. It's been discarded, as it should be. Second, a new term has been developed. "Ribozymes" are RNA molecules with enzymatic properties, or RNAs that, like those in ribonuclease P, make up part of an enzyme that contains other compounds as well. Finally, eyes have been opened everywhere to the notion that RNA can play biochemical roles as complex and important as catalysis.

In 1990, Tom Cech and Sidney Altman shared the Nobel Prize for their demonstration that RNA could act as an enzyme. The search for ribozymes has now widened to the point where RNA can be suspected of catalytic properties in any RNA-protein complex, and researchers are revising their thoughts about the evolution of life on Earth.

Ribosomes and the Primitive Soup

Two longstanding mysteries may eventually be solved by invoking ribozymes. The first involves one of the most important structures in the cell, the ribosome. Ribosomes are the factories where polypeptides are assembled following instructions encoded in mRNA. Ribosomes contain 3-4 RNA molecules and 70-90 proteins. For more than 30 years biochemists have tried to find which of the many proteins contain the enzyme activity that forms the peptide bond between two amino acids. The "enzyme" responsible for this activity has always been called peptidyl transferase, but it has never been isolated. Attempts to pinpoint which of the many ribosomal proteins might be peptidyl transferase have always failed.

Finally, in 1992, a group of researchers led by Harry Noller of California carried out the simple experiment of stripping ribosomes of all their proteins. To their surprise, the stripped ribosome, which contained only rRNA, could still catalyze peptide bond formation. As you might suspect, the researchers concluded that peptidyl transferase was, in fact, a ribozyme. Their results have not yet been repeated, and there's still a chance that rRNA is not involved in peptidyl transferase, but it certainly looks as though rRNA is much more than the unimportant scaffold upon which a slew of important proteins are hung.

Ribozymes have also revolutionized thinking about the evolution of the first life forms. For years, researchers have wondered if it was possible that the first self-replicating molecules could have been

nucleic acids, like DNA. DNA replicates, of course, but only with the aid of a complex group of proteins. Researchers realized that they had a classic chicken-or-the-egg problem. Proteins cannot exist without DNA to specify their construction, and DNA cannot replicate without proteins.

The catalytic properties of RNA change the rules of the game. RNA molecules can catalyze their own splicing, and there is even evidence that they can undergod self-catalyzed replication in the absence of proteins. This has allowed investigators to speculate that small RNA molecules may have led the biochemical revolution that resulted in the evolution of the first living cells. RNA seems to be able to direct the synthesis of polypeptides, to direct its own replication, and to catalyze a wide variety of other chemical reactions as well. In the minds of many researchers, this makes it a perfect candidate as the driving force behind the evolution of life. All this, from one lab's "failure" to find that enzyme that they knew *had* to be there to splice *Tetrahymena* rRNA.

Important Teaching Points

1) The first lesson to be drawn from the ribozyme story is the obvious one, namely that RNA can act as an enzyme. Although the number of well-studied ribozymes pales in comparison next to the enormous inventory of protein enzymes, the field is still developing. Students should be prepared for more discoveries in the years ahead, especially those that may relate to basic cellular processes including replication, transcription, and translation.

2) Cech's Colorado group expected to find an enzyme at the heart of the rRNA splicing reactions they investigated. When they did not, they could easily have assumed that their results were mistaken, and simply tried to work on something else. However, they had the courage to believe their own, puzzling results. That courage led directly to one of the most important biochemical discoveries of the 20th century. Students should take this as a case in point that scientists must be prepared to sieze upon results that simply don't make sense. That's where the next great breakthrough may be.

3) At first glance, it might make little sense to waste time, effort, and money studying something like RNA splicing in *Tetrahymena. Tetrahymena* don't get cancer, they don't cause disease, and they're not very much like people. As we've seen, Cech's lab chose to study these organisms not because they were intrinsically interesting, but because they promised to be a good "system" to investigate a basic biology problem, the question of RNA splicing. As it turns out, their choice of organism was brilliantly correct. They were not only able to determine the splicing mechanism, but their work led to the discovery of a whole new biochemical mechanism -- one that applies to humans as much as it does to every other organism.

Suggestions for Further Reading:

Unfortunately, the ribozyme story has not been written up as extensively as it should be. Here are a few source articles that describe the initial scientific reaction the discovery of catalytic RNA.

R. Lewin (1982) RNA can be a catalyst. Science *218:* 872-874.

R. Lewin (1984) First true RNA catalyst found. Science *223:* 266-267.

A. Scott (1984) RNA can be a catalyst too. New Scientist (March 8, 1984) p. 21.

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