First True RNA Catalyst Found

Presaged by an earlier discovery, the first RNA molecule that acts as a true catalyst has now been found. There will be others

When, a little more than a year ago, Thomas Cech and his colleagues at the University of Colorado reported that a ribosomal RNA precursor molecule in the ciliated protozoan *Tetrahymena thermophila* could perform a biochemical reaction upon itself—that the RNA could act like a catalyst in the absence of protein—it was described by one commentator as "one of the most exciting discoveries of the decade." Here was one of the great assumptions of biology—that only proteins can act as biological catalysts—tumbling before irrefutable evidence.

Remarkable though the observation was, some observers noted that the *Tetrahymena* ribosomal RNA could not be described as a true catalyst, because it failed on one crucial criterion. A true catalyst, as every chemist learns, must emerge unchanged from the reaction. In Cech's case the ribosomal RNA self-splices a 413 nucleotide intervening sequence and joins the two resultant fragments together to give a mature molecule. In other words, the RNA is changed in the reaction and can no longer catalyze splicing. Ergo, it is not a true catalyst.

In spite of this shortcoming, Cech's result constituted such a convincing assault on biologists' preconceptions that it encouraged a belief that sooner or later a true RNA catalyst would be found. This has now been done, and it marks the culmination of a sometimes tough and bitter scientific crusade for one of the researchers involved.

Sidney Altman and his colleagues at Yale in collaboration with Norman Pace and co-workers at the National Jewish Hospital in Denver report in the current issue of *Cell** that the catalytic activity of the enzyme ribonuclease P is carried out by RNA, not by protein. The enzyme, which cleaves precursor transfer RNA molecules to produce mature transfer RNA's, is composed of about five times RNA to protein by weight. The Yale and National Jewish Hospital teams have demonstrated that, stripped of its protein, the RNA component can perform the catalytic activity of the enzyme whereas the protein alone cannot.

Altman has been doing battle with ribonuclease P for more than a decade and with the rest of the scientific community over what he discovered for a little less. The enzyme, which has been found more or less wherever it has been sought in lower and higher organisms, proved to be very difficult to isolate and somewhat tricky to handle. The main problem with its isolation was that, contrary to all expectations, the complex was mostly composed of RNA associated with a little protein, as Ben Stark experienced through arduous work in the Yale laboratory.

The crucial experiments that were to separate ribonuclease P from all known enzymes involved the destruction of its catalytic activity by treatment with a nuclease that cleaves RNA. Stark's experiments, which were done through 1975–1976, indicated to Altman that the combination of protein and RNA was essential for enzyme activity. Further work, principally by Richard Kole in the Yale lab, showed that the catalytic activity could be abolished or restored through dissociation and reassociation of the components parts. These observations convinced Altman that his initial conclusion was correct, and he began to promote the idea publicly thereafter.

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The response can be described as, at best, unenthusiastic. The idea was apparently judged to be so off the wall that it did not bear serious consideration: enzymes were proteins, not RNA molecules, everyone knew that.

Altman persisted in his assertion while almost everyone else persisted in their indifference, until very recently when the weight of evidence could no longer be ignored. Because of the manner in which perceptions shift in science, when Cech and his colleagues made their even more radical claim in 1982—that RNA alone could perform a quasi-catalytic reaction—they faced no hostility. The barriers had been broken down and the path beyond was relatively smooth. And it must be true that, in its turn, Cech's discovery of self-splicing in *Tetrahymena* ribosomal RNA will ease the acceptance of RNA as a true catalytic molecule in ribonuclease P. Here, the wheel of fate has turned full circle.

Escherichia coli has been the principal source of ribonuclease P for Altman, whereas Pace's interest is in the *Bacillus subtilis* enzyme. The two groups began collaborating at the beginning of last summer, partly to test whether heterogenous complexes—that is, for instance, the *E. coli* RNA component combined with the *B. subtilis* protein—would process transfer RNA precursors normally. They did. During the collaboration Katheleen Gardiner, in Pace's laboratory, increased the magnesium concentration of the reaction medium in an attempt to boost activity. This turned out to be a crucial modification, not because it did indeed enhance the normal reaction but because it is only with high magnesium levels that the RNA alone will be catalytic.

So, serendipitously, one of the control experiments that contained RNA alone at high magnesium displayed enzyme activity, and the data then began to pour out. Cecilia Guerrier-Takada in Altman's laboratory had been poised to carry out a large series of other investigations with the enzyme and its various components, and so was well placed to concentrate all her skills and material on this new and more interesting avenue of work. Most recently, the Yale group has ruled out the remote possibility that the catalytic activity of the RNA is not the result of contamination with protein by the expedient of testing RNA that has been transcribed in vitro, which obviates any chance of associated protein (see page 285 of this issue).

*C. Guerrier-Takada, K. Gardiner, T. Marsh, N. Pace, S. Altman, *Cell* **35**, 849 (1983).

Although the RNA components of the *E. coli* and *B. subtilis* enzymes are demonstrably different—a gene probe from *E. coli* does not hybridize with the *B. subtilis* genome—each can cleave the natural transfer RNA substrates of the other. Differences in primary sequence of the RNA molecules—both of which measure around 370 nucleotides long—does not necessarily translate to differences in secondary and tertiary structure, however. Pace is especially interested in comparing secondary structure arrangements in the RNA from several organisms to see which aspects are conserved: these are likely to be the functional parts. He expects to have the primary sequence of the *B. subtilis* RNA within weeks and a second one soon thereafter. Both Pace and Altman plan to modify the RNA's—by insertions, deletions, and specific mutations—as a way of dissecting the functionally operative domains of the molecules.

In addition to cleaving a tail section from transfer RNA precursors, ribonuclease P in *E. coli* is also responsible for a similar processing reaction on an enigmatic molecule called 4.5*S* RNA. In this reaction only the ribonuclease P RNA from *E. coli* will work in vitro, not that from *B. subtilis*, and only then when it is combined with a protein component, which can be from either organism. The catalytic activity must be a little different in this case, as well as there being a clear species specificity with respect to the RNA component of the enzyme.

RNA molecules are highly flexible entities, especially when compared with the relatively rigid strands of DNA, and have a greater facility than proteins for action over large distances. So, far from being the dumb slaves of the all mighty DNA, RNA molecules have great functional potential.

This latest discovery with ribonuclease P will undoubtedly spur the systematic search for further examples of RNA as catalyst, of which there are many potential candidates. In addition to the well-established occurrences of protein and RNA combinations—for instance, in the ribosome, several classes of ribonucleoprotein particles in the nucleus and cytoplasm, and the recently discovered signal recognition particle—in which the RNA might possibly be playing more than a passive structural role, there are scores of small RNA molecules in all cells for which no function has yet been identified.

Furthermore, there are at least two cases of enzyme activity that apparently require the presence of an RNA molecule. One, reported last October by Max Birnstiel's group at Zurich University, Switzerland, implicates a 60 nucleotide RNA in the processing of a histone messenger RNA in a sea urchin species. Another, worked on by a Russian group, involves the modification of the carbohydrate amylose by an RNA-containing enzyme. If this latter case were to be confirmed as truly catalytic RNA, it would be the first example of an RNA catalyst effecting a chemical modification in a non-RNA substrate.

For those interested in the origin of life, the existence of RNA catalysts offers an intriguing glimpse of a former, more primitive age when the full range of metabolic and genetic machinery had yet to evolve. If, as now seems certain, RNA molecules can perform a range of catalytic functions in addition to being carriers of information, the old origins conundrum of "protein before DNA or DNA before protein?" is mercifully eschewed.—**Roger Lewin**

One Billion Transistors on a Chip?

The annual rate of increase in numbers of transistors on a chip is slowing as theoretical limits are neared, but there may be a billion by the year 2000

It could happen by the turn of the century, according to James Meindl, director of Stanford University's Center for Integrated Systems. Meindl's forecast came in the opening session of the 3-day International Electron Devices Meeting, held in Washington, D.C., early in December. For comparison, the most densely packed integrated circuits at present cram about 600,000 transistors onto a silicon chip about 6 millimeters on a side. Moreover, these integrated-circuit chips, random access memories that store 262,144 binary bits of information (so-called 256K RAM's), will not be commercially available for another year or two.

Projection of the future course of semiconductors has become a liturgical requirement of integrated circuit meetings since Gordon Moore of the Intel Corporation formulated "Moore's law" in the mid-1970's. Moore observed that the number of transistors on a chip had been roughly doubling each year since Texas Instruments and Fairchild Semiconductor independently developed the integrated circuit in 1959. From 1973 to the present, the rate of growth has been slightly lower, the number of transistors per chip increasing by a factor of 4 every 3 years. A continuation of this trend would find integrated circuits of over 10 billion transistors by the year 2000.

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Meindl's message, then, is that further moderation of the growth curve is in store. Depending on certain assumptions pertaining to the fabrication technology, the number of transistors per chip may climb to a number ranging from "only" several hundred million to about 1 billion in the next 16 years. The reason for the anticipated decline in rate is that engineers are approaching a number of theoretical and practical limits on the minimum size of transistors. Their situation is like that of a football team that finds it harder to advance the ball as it nears its opponent's goal line because there is less room for maneuvering.

Meindl calls this future era ULSI, for ultralarge-scale integration, as opposed to the current VLSI or very-large-scale-integration epoch. The upcoming generation of 256K RAM's have minimum feature sizes ranging from 1.3 to 2.5 micrometers. The minimum feature size is usually defined as the average of the width of the electrical conductors that connect transistors and the spacing be-