CLASSIC EXPERIMENT 8.1

CATALYSIS WITHOUT PROTEINS—THE DISCOVERY OF SELF-SPLICING RNA

K. Kruger et al., 1982, *Cell* **31**:147

For biological systems to function, countless reactions must be catalyzed. These duties are carried out by enzymes, biological macromolecules that readily enhance reaction rates yet remain unconsumed by the reaction. For many years only proteins were believed to possess sufficient diversity of functional groups to catalyze the myriad reactions necessary to sustain life. Then, in 1981, Thomas Cech reported that, in at least one case, RNA could do the job.

Background

In eukaryotes and many viruses, genes contain sequences that are initially transcribed, then subsequently removed from RNA as they are not part of the actual coding sequence. These sequences are known as intervening sequences (IVS) or introns. IVS are removed from precursor RNA by a biological process known as splicing. While investigating the splicing of precursor ribosomal RNA (pre-rRNA) genes, transcribed from rRNA genes, Cech made his critical discovery that RNA exhibited catalytic activity.

Cech wanted to understand the molecular components of RNA splicing. Rather than examing complex eukaryotic genes, he chose a simple model system, rRNA genes from the ciliated protozoan *Tetrahymena thermophilia*. By isolating *Tetrahymena* nuclei, Cech and his coworkers developed a system in which pre-rRNA gene splicing could be studied in vitro. The purified nuclei could perform both transcription of rRNA genes and processing of the large pre-rRNA that initially is formed. Using this system, Cech found that during synthesis of 26s rRNA in *Tetrahymena*, a 0.4-kb IVS is removed. The next step was to perform pre-rRNA splicing with nuclear extracts, with an eye toward purifying the enzymes that catalyzed the splicing reaction. Although Cech succeeded in this goal, he could have never guessed how the catalysis was taking place.

The Experiment

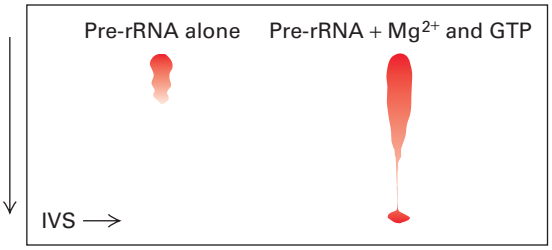
Cech's plan was to use the in vitro splicing system to purify the RNA-splicing enzymes, a common experimental approach for dissecting complex molecular processes. First, the reaction is characterized in a cell-free system, in this case purified nuclei. Then a means to purify the reaction substrate is developed. In the case of the *Tetrahymena* rRNA splicing this was relatively easy, because the full-length rRNA (pre-rRNA) transcripts were abundant in *Tetrahymena* nuclei and readily purified. Finally, cellular extracts are added back to reconstitute the activity being studied. Since the RNA splicing activity was known to take place in the nucleus, Cech used nuclear extracts. In fact, he could readily see splicing when nuclear extracts were added to rRNA transcripts in a splicing cocktail composed of Mg^{2+} and guanosine triphosphate (GTP). Unexpectedly, splicing also occurred when rRNA transcripts were incubated in the splicing cocktail in the absence of a nuclear extract. This activity was reproducible, leaving open two possibilities: Either the purified pre-rRNA remained associated with an enzyme (i.e., a protein contaminant) or the pre-rRNA was catalyzing its own splicing.

The first step in determining which possibility was correct was to see if the rRNA transcripts were truly devoid of protein. Because proteins are notoriously fragile biomolecules, whose activity is easily destroyed by heat, chemicals, and proteolytic enzymes, Cech subjected the rRNA transcripts to numerous treatments known to degrade proteins: first, boiling to promote heat denaturation; then, extraction with organic solvents to promote chemical denaturation; finally, incubation with a variety of proteases to promote enzymatic degradation. Still, the pre-rRNA retained its splicing activity. These results strongly suggested that *Tetrahymena* pre-rRNA is indeed self-splicing. But a more definitive experiment was needed to convince other researchers that the transcripts were uncontaminated by protein and possessed inherent catalytic activity.

Fortunately, the *Tetrahymena* pre-rRNA could be produced in vitro using purified RNA polymerase from *E. coli*. Transcription of the *Tetrahymena* rRNA gene with a polymerase from a different organism would eliminate the risk that the RNA remained associated with a *Tetrahymena* enzyme. In this system, the only enzyme ever associated with RNA would be *E. coli* RNA polymerase, which was readily removed by extraction with organic solvents. Using this system, Cech carefully synthesized the *Tetrahymena* pre-rRNA, removed the polymerase, and purified the transcripts. When he incubated this in vitro synthesized pre-rRNA in the splicing cocktail, analysis of the products showed that once again, the IVS was removed from the precursor (see Figure). This experiment proved that the *Tetrahymena* pre-RNA was self-splicing, catalyzing the removal of the IVS without the aid of any protein.

Discussion

Cech called his self-splicing RNA a "ribozyme," implying that it was an



Demonstration that *Tetrahymena thermophilia* pre-rRNA can self-splice. Radioactively labeled pre-rRNA was synthesized in vitro using *E. coli* RNA polymerase and then incubated in neutral buffer or in the presence of Mg^{2+} and GTP, necessary cofactors for the splicing reaction. Depicted here is an autoradiograph of the electrophoresed samples revealing the spliced-out IVS in the sample containing splicing cofactors. [Adapted from Kruger et al., 1982, *Cell* **31:**147.]

RNA enzyme. Although the demonstration of self-splicing RNA was readily accepted by the scientific community, many were skeptical about the notion that RNA was a true catalyst. In subsequent studies, however, Cech was able to engineer the *Tetrahymena* rRNA IVS such that it could be used as an enzyme, splicing one RNA molecule, then turning over to splice others. This convinced even the skeptics that RNA can have true catalytic activity. Soon other self-splicing RNAs and other catalytic RNAs were identified. RNA catalysis has become a field of study unto itself, with research on the use of catalytic RNA in both laboratory and medical settings. Furthermore, the ability of RNA to catalyze biological reactions has evolutionary implications. It is now conceivable that primordial organisms contained only RNA and later evolved the more complex system of proteins. For his pioneering work on RNA catalysis, Cech was awarded the Nobel Prize in Chemistry in 1989.