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FIRST DIRECT EVIDENCE OF GENETIC SPLICING PHENOMENON SUPPORTS THE ROLE OF CATALYTIC RNA IN EVOLUTION

For Immediate Release

December 11, 1989

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The first direct evidence of a genetic cut-and-paste phenomenon used for rapid protein development complements the work of two 1989 Nobel Prize winners who demonstrated the catalytic properties of RNA, an intermediary genetic material. Catalytic RNA research has captured the imagination of chemists and molecular biologists because findings are consistent with an "RNA World" theory that suggests RNA -- rather than DNA -- might have been the original genetic material on earth.

Since 1978, scientists have suspected that nature uses large sections of existing genes as "modular building blocks," by shuffling the coding regions called exons which express protein characteristics. Until now, however, evidence of this exon shuffling was largely circumstantial.

Researchers at the Georgia Institute of Technology and the University at Albany, State University of New York (SUNY Albany) recently observed actual exon shuffling in two genes which nature routinely copies or transcribes into catalytic RNA. Exon shuffling is believed to occur only rarely, but the Georgia Tech/SUNY Albany team says this short-cut production method may have been far more prevalent during an earlier stage of evolution, to speed the development of new proteins.

"New genes can evolve rapidly by bringing existing exons of different genes together," said Biology Professor Dr. Dwight H. Hall of Georgia Tech. "It's a mixing and matching of genes; taking what's useful from perhaps several different genes and using this information as building blocks, instead of starting from scratch with the individual components."

Pondering an RNA World

Hall and Dr. David A. Shub of SUNY Albany studied DNA to unearth direct evidence of spontaneous exon shuffling. Since the DNA they studied is transcribed into catalytic RNAs, their work is consistent with the RNA world theory.

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Today, proteins evolve from a genetic code in DNA, the 'genetic fingerprinting' material that determines the characteristics of an organism. The DNA is transcribed into RNA before being translated into a protein. As part of this process, scientists have learned, some RNAs splice together genetic coding regions (exons) and cut out the non-coding sections called introns, or interruptions in the coding sequence.

For years, scientists believed that RNA had to interact with a protein to initiate the chemical reaction that causes splicing. But in self-splicing RNA, discovered by Dr. Thomas R. Cech, exons are spontaneously joined when the intron folds into itself, thus splicing the exons together. Cech and Dr. Sidney Altman shared the 1989 Nobel Prize for chemistry after they independently observed catalytic RNA.

Biologists have long wondered why introns are retained and transcribed only to be discarded. Noting that a number of RNAs are self-splicing, some scientists think RNA may have been gradually modified and refined, through an evolutionary process, to produce DNA.

"The kinds of introns that are self-splicing probably represent a primitive form of intron that existed in an early evolutionary time, perhaps when life had not even diverged into the different kingdoms," said Shub. "In our work, we might be looking at a molecular fossil of an intermediary time in evolution when genes were largely made of DNA but the introns within them were all of the self-splicing variety."

The concept of exon shuffling, which offers a rationale for the retention of the apparently useless information in introns, was first outlined in 1978 by Nobel Prize-winning scientist Walter Gilbert, explained Shub, a biology professor and director of the Center for Molecular Genetics at SUNY Albany.

In the past, researchers pondered the concepts of exon shuffling and an RNA World simply by analyzing similar portions of different proteins which appeared to be the result of exon shuffling. "This is the first real direct evidence which makes exon shuffling a much more tenable hypothesis," Hall noted.

If the cut-and-paste theory of evolution is accurate, Hall added, it could explain why seemingly non-essential "junk" exists between exons in the form of introns; these non-coding areas might be attractive evolutionary targets for recombination.

Proving the Exon Shuffle

Hall and Shub examined exon shuffling in two genes that code for enzymes: thymidylate synthase (td), and a small subunit of ribonucleoside diphosphate reductase (nrdB). These genes may be found in a common bacterial virus known as T4, which exists in the intestine. (The T4 virus contains about 200 genes, including self-splicing td and nrdB genes.)

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Samples of normal or "wild-type" td and nrdB genes found in T4 were compared with mutant specimens to determine the position of exons, before and after a shuffle.

The Georgia Tech/SUNY Albany team observed the spontaneous occurrence of two hybrid introns, from both nrdB and td. These hybrid introns occupied a space between an exon from td and another exon from nrdB.

In one case, the resulting hybrid was functional; that is, the intron was able to splice itself by folding up to unite a pair of exons, Hall reported.

Before comparisons could be made between wild-type and mutant genes, two sets of td and nrdB genes had to be selected using two different techniques. Shub and Hall didn't perform additional experiments to detect the specimens; instead, they used genes which had already been isolated during previous research projects.

The first mutant specimens of td and nrdB were isolated using a "genetic engineering trick" which involves duplicating one area of the chromosome, which is made up of many genes, thus forcing it to correct its length by spontaneously deleting a portion of coding. The double-stranded DNA from the T4 virus will automatically compensate for any duplication of coding by dropping a comparable section to maintain a consistent length, Hall explained.

A second set of spontaneous deletion td and nrdB genes was detected through a procedure called "folate analogue resistance." Folate analogues are inhibitory drugs that prevent the growth of wild-type T4. However, Hall knew that drug-resistant mutants would thrive in the presence of the drug, so they were easily detected in a folate analogue mixture.

Once the specimens were selected, Hall and Shub used the T4 virus containing td and nrdB genes to infect intestinal bacteria cells known as Escherichia coli (E. coli). After seven minutes, the cells were chilled and disrupted to extract RNA. Next, the RNA was analyzed and converted to a DNA sequence using a method known as reverse transcription. Finally, the samples were studied through electrophoresis to determine their coding sequence. This procedure involves applying electricity to a gel mixture containing the genetic material, which has been labeled with radioactivity. Since the current separates coding regions, they can then be exposed on X-ray film.

Hall and Shub published their work in a recent issue of Nature.

EDITOR'S NOTE: Please see back page for graphic illustrations of concepts described in this article. For black-and-white or color photographs, call the contacts listed on page one. Copies of published results are also available.

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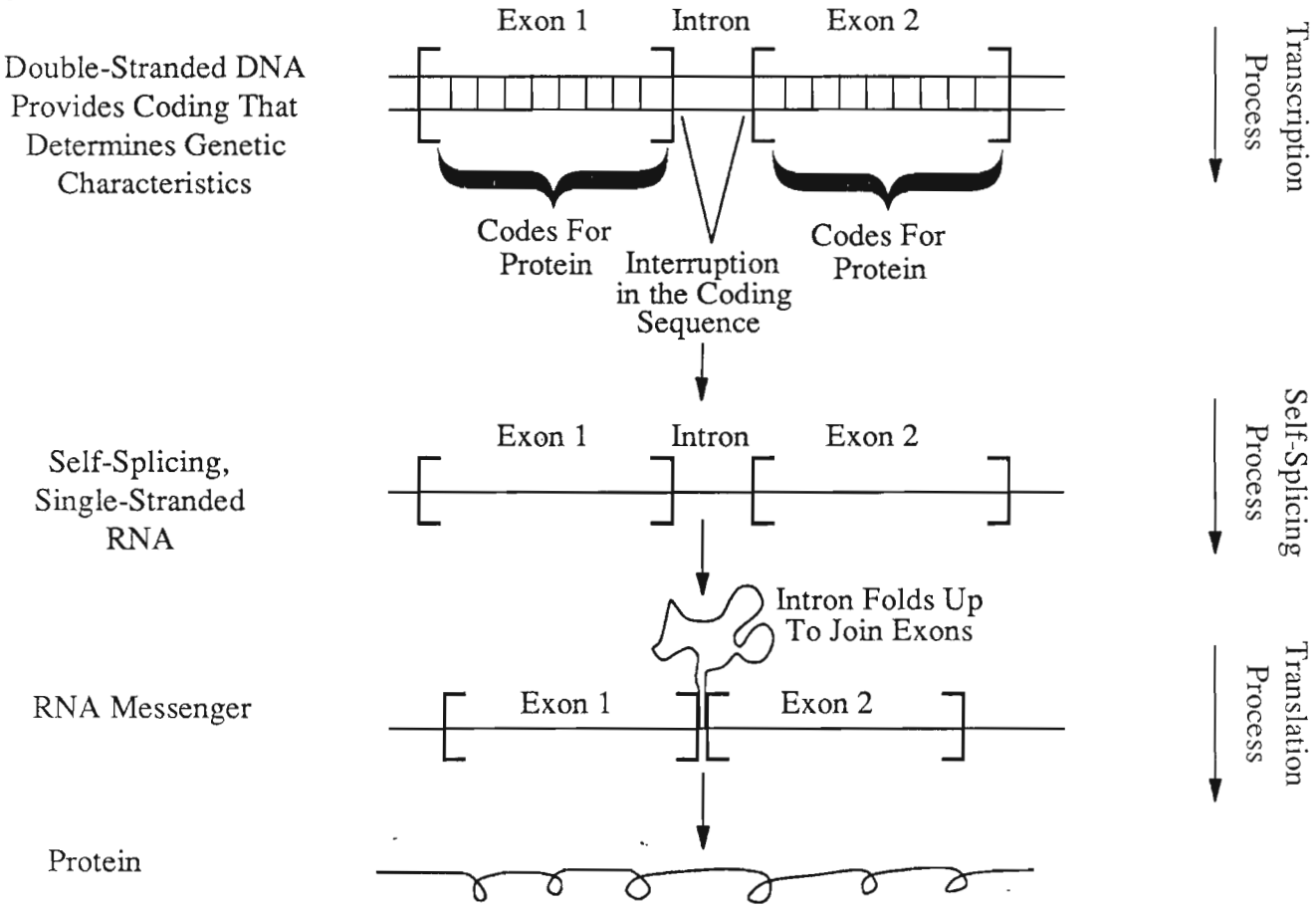
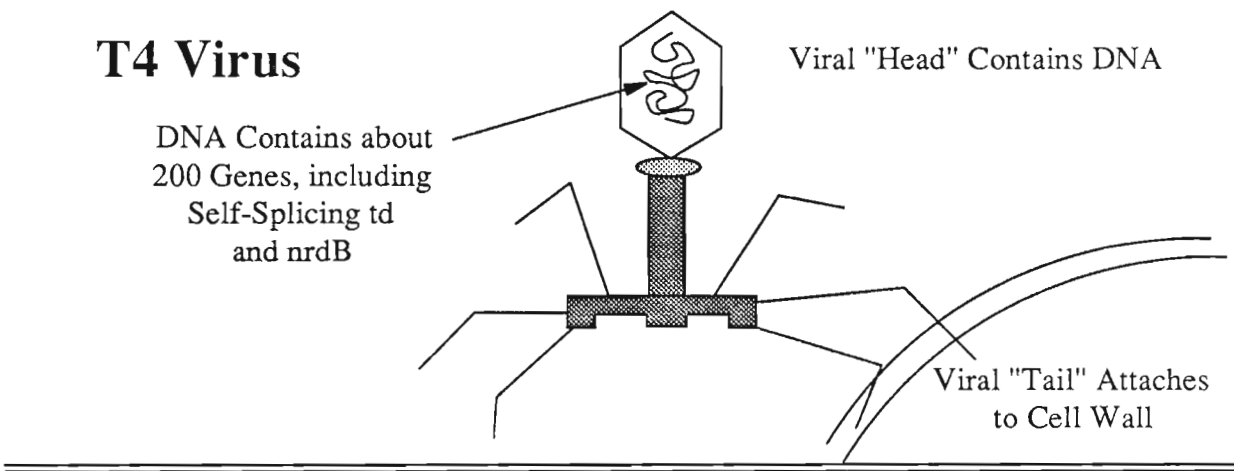
Protein Development

Typical Split-Gene



T4 Virus



Definitions: Chromosome - A long, thread-like body inside plant & animal cells which contains DNA, the genetic coding material that determines protein characteristics.

Each gene occupies a small section of a chromosome.