

Biography of Jennifer A. Doudna

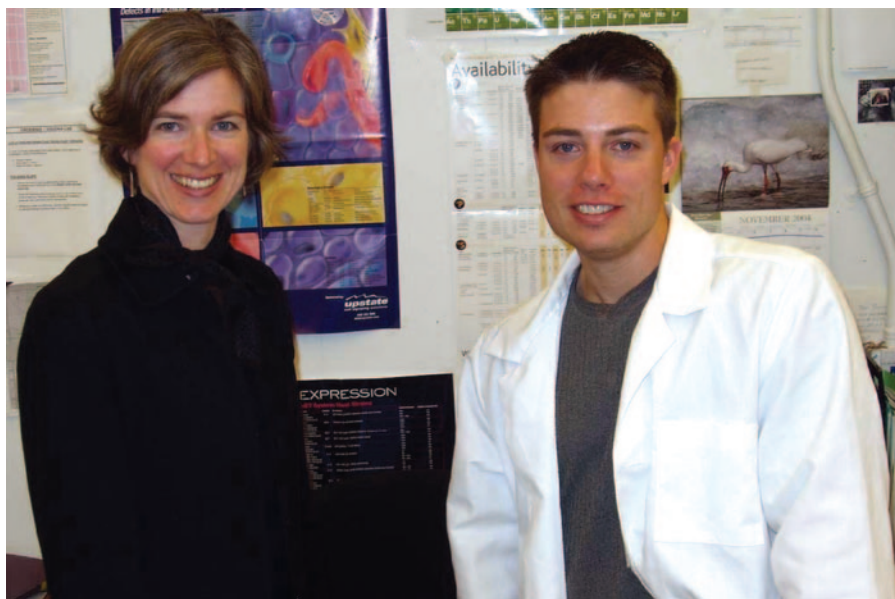
In the central dogma of molecular biology, DNA is transcribed into RNA, which then is translated into protein. Although RNA may be considered simply an intermediary between these two important biological molecules, RNA is much more than just a recipe for making proteins. In the 1980s, researchers showed that certain RNA molecules function as enzymes, a role previously attributed solely to proteins. Jennifer A. Doudna, Ph.D., Professor of Molecular and Cell Biology and Chemistry at the University of California, Berkeley, has devoted her scientific career to revealing the secret life of RNA. Using structural biology and biochemistry, Doudna's work deciphering the molecular structure of RNA enzymes (ribozymes) and other functional RNAs has shown how these seemingly simple molecules can carry out the complex functions of proteins.

In two landmark studies, Doudna and colleagues solved the crystal structures of two large RNAs, the P4-P6 domain of the *Tetrahymena thermophila* group I intron ribozyme (1) and the hepatitis delta virus (HDV) ribozyme (2). By determining their molecular structures, her work has advanced the understanding of RNA's biological function. In her Inaugural Article published in this issue of PNAS (3), Doudna describes how a special piece of hepatitis C viral RNA, called the internal ribosome entry site (IRES), hijacks the host cell's machinery and induces it to churn out viral proteins.

Among Doudna's numerous awards and accomplishments are the Searle Scholar Award (1996) and the National Academy of Sciences Award for Initiatives in Research (1999). She is also an investigator with the Howard Hughes Medical Institute and an American Academy of Arts and Sciences Fellow. In 2002, Doudna was elected to membership in the National Academy of Sciences for her contributions to the field of biochemistry.

Erupting Scientific Interests

Doudna grew up in Hawaii amidst the volcanoes, lush tropical forests, and remote beaches of Hilo. These natural wonders instilled in her an awe and appreciation of nature. Although her immediate and extended family had no scientists, Doudna first became interested in science in high school when she took her first chemistry class and participated in a science seminar series highlighting the chemistry of biological



Jennifer Doudna in the laboratory with postdoctoral associate Richard Spanggord.

systems. Her parents, both academics in the humanities with avid interests in astronomy, geology, and evolution, encouraged her interests. They provided Doudna with science books, museum visits, and her first "hands-on" science experience—a summer studying worms and mushrooms in the laboratory of professor and family friend Don Hemmes, at the University of Hawaii (Hilo). After reading *The Double Helix*, James Watson's account of his and Francis Crick's discovery of the structure of DNA, Doudna was hooked on science and desired to delve deeper into the mysteries of the life sciences.

To indulge her scientific curiosity, Doudna studied chemistry at Pomona College (Claremont, CA) where she met several people who had a profound impact on her research career. These included chemistry professors Fred Grieman, whose passion for quantum mechanics was "infectious," and Corwin Hansch, whose intensity and love for research was "inspirational." Doudna began her first scientific research at Pomona, working in the laboratory of Sharon Panasenko, her undergraduate advisor. Panasenko was not only a superb scientist, said Doudna, but also led by example, showing that a woman could be successful in what some perceive as a male-dominated academic world. "It's a challenging job, especially for women," says Doudna. "The further along I get in my career, the more I see how important it is for young women to

have supportive female mentors." Doudna feels fortunate to have had a strong female role model like Panasenko to help guide her early in her career.

RNA Exploration Begins

After earning her bachelor's degree in chemistry from Pomona in 1985, Doudna went on to pursue her biochemistry doctorate with Jack Szostak at Harvard University (Cambridge, MA). During this time, Doudna's fascination with RNA began to blossom. "Up until the late 1980s, it was known that RNA was involved in protein synthesis, but the discovery that RNA could have catalytic activity really revolutionized the whole field," recalls Doudna. This realization that RNA might have much greater functional activities than its role as messenger RNA (mRNA) or as part of the ribosome provided the impetus for Doudna's subsequent research career. With Szostak, Doudna made her first mark on the RNA field. Doudna and Szostak (1) reported the reengineering of an RNA self-splicing intron into a ribozyme, capable of copying an RNA template. "It was exciting for us because it suggested that RNA could function as a polymerase," Doudna says.

This is a Biography of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 16990.

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Graduating with her doctorate in 1989, Doudna remained in Szostak's laboratory as a postdoctoral fellow to continue her studies on self-replicating RNAs. "In the course of this work, I became curious about what the RNA structure might be that would allow it to have this kind of activity," says Doudna.

This curiosity eventually led Doudna to the University of Colorado (Boulder) and the laboratory of Thomas Cech, who received the 1989 Nobel Prize in chemistry for discovering the catalytic properties of RNA. As a research fellow in Cech's laboratory, Doudna began crystallizing RNA molecules in hopes of obtaining a molecular portrait of these unique structures. According to Doudna, Cech is a deeply insightful scientist who had assembled an outstanding laboratory research team. "The highlight of this time period was the late-night discussions with members of the lab and the occasional intense brainstorming sessions with Tom," Doudna says.

Characterizing Ribozyme Structures

In 1994, Doudna joined the faculty at Yale University (New Haven, CT) as an assistant professor. She rose rapidly through the ranks, becoming Henry Ford II Professor of Molecular Biophysics and Biochemistry in 2000. Early in her tenure at Yale, Doudna and Cech published the crystal structure of the P4-P6 domain of the *Tetrahymena thermophila* group I intron ribozyme (4). "That was the first time anyone had seen what a large structured RNA looked like other than tRNA," Doudna says. This study directly revealed that RNA, rather than being a spaghetti-like molecule, could have a defined shape. "This P4-P6 domain structure showed us how RNA is able to pack helices together to form a three-dimensional shape," she says, "which is much more reminiscent of what we see in proteins than anybody had previously been aware of for RNA."

In the studies that followed, Doudna, Jamie Cate, and Raven Hanna found that a core of five magnesium ions clustered in one region of the P4-P6 domain, forming a nucleus around which the rest of the structure could fold (5). "We presented that as analogous to what happens in proteins, where protein structures typically fold around a hydrophobic core," she says. "Here the core is chemically different, but the principle of folding is similar."

Also in the P4-P6 crystal structure, Doudna observed several examples of a particular motif—abundant adenosines in unpaired regions of the RNA structure. By using various mutagenesis

experiments, Doudna, along with Liz Doherty and Rob Batey, showed that this motif was the most critical interaction for allowing the RNA to form the structure that it does (6). Around the same time, her colleague at Yale, Thomas Steitz, reported the existence of an abundance of the same motif in the ribosome (7). "Even in a very large RNA, a motif that was observed in the P4-P6 domain turns out to be probably the most important motif, energetically, for folding the RNA," says Doudna. Her work on the P4-P6 domain began to illustrate the structural similarities between ribozymes and protein enzymes.

"Can we get enough information so that we can understand the chemical basis for RNA's many biological functions?"

In a series of studies similar to the P4-P6 studies, Doudna examined a type of nonenzymatic RNA found within an RNA-protein complex called the signal recognition particle (SRP). SRP is a macromolecular machine that recognizes proteins leaving the ribosome and shunts them across the membrane of the cell or the endoplasmic reticulum. Doudna became "interested in [SRP] a few years ago because, as an RNA-focused lab, we wondered why this RNA is so important for protein recognition and why it had been so highly conserved in evolution."

Doudna and postdoctoral fellow Robert Batey went on to characterize the crystal structure of the signal-recognizing domain of SRP (8). In addition to the basic structure, Doudna, Batey, and Brian Rha conducted an *in vivo* experiment using a strain of bacteria in which portions of SRP sequence were manipulated. They found that only the most conserved part of the structure was necessary to support growth (9,10). "Analogous to what we had been able to do with the P4-P6 structure," says Doudna, "we were able to use the crystal structure of the core of the SRP to direct biochemical studies to test what the most important interactions were energetically for holding that structure together."

Investigating Viral RNA

Although Doudna has a strong interest in the basic structure and function of RNA molecules, much of her current work involves viral RNAs. Determining how these RNAs function could lead to strategies to combat a host of viral diseases. Doudna's laboratory has determined how the small, self-cleaving RNA found in the hepatitis delta virus (HDV) is able to function. As a human pathogen, HDV is only coinfectious in patients who already have a hepatitis B virus infection; patients with HDV superinfection develop a more severe form of hepatitis.

Doudna, Adrian Ferre-D'Amare, and Kaihong Zhou published the crystal structure of this viral RNA in 1998 (2). Based on this structure, they proposed that the HDV ribozyme uses a cytidine base in the RNA to shuttle protons during the reaction, a mechanism remarkably similar to protein ribonucleases. "The surprise was that RNA might be able to use a mechanism that is much more similar to proteins than had been appreciated before," says Doudna. In 2004, Doudna, Zhou, and Ailong Ke published additional information on the functional characteristics of this viral RNA (11).

Another focus of Doudna's laboratory involves a segment of viral RNA called the internal ribosome entry site (IRES). According to Doudna, IRES is a "pretty amazing structure that's basically able to grab the ribosomes of infected cells and hijack them for making viral proteins." Her team is studying this in the hepatitis C virus (HCV), but the mechanism may be common to a number of viruses, including poliovirus. "I think this is the project in the lab that has the greatest potential to lead to something that might have an impact on human health," Doudna says. In a series of experiments, Doudna and Jeff Kieft determined that HCV IRES RNA actually has a three-dimensional structure responsible for its activity (12, 13).

In a collaboration with Joachim Frank at the State University of New York in Albany, Doudna characterized HCV IRES RNA interaction with the small subunit of the ribosome by using cryo-electron microscopy (EM) (14). The cryo-EM structures revealed that a mutant form of IRES was unable to induce a conformational change in the 40S ribosome that normally occurs when it binds to wild-type IRES RNA. This conformational change in the 40S subunit of the ribosome occurs in the region where the ribosome binds to the mRNA strand. "Based on the cryo-EM structures, the IRES RNA might actu-

ally be functioning like a C-clamp on the ribosome to physically clamp the ribosome onto the viral mRNA in the correct place to initiate translation,” says Doudna.

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In 2002, Doudna made another cross-country move to accept a faculty position at the University of California, Berkeley, as Professor of Biochemistry and Molecular Biology. Doudna, her husband, and their 2-year-old son, whom she calls “the biggest experiment I ever did,” are closer to their extended families and to a resource essential to both of their research—the synchrotron at the Lawrence Berkeley National Laboratory.

At Berkeley, Doudna has continued to study IRES RNA. A curious aspect of IRES RNA is that it requires only a few of the initiation factors normally used to begin translation. In her Inaugural Article (3), Doudna, Ji Hong, and Chris Fraser extend previous findings to suggest a reason why mutant IRES RNA stalls at particular points in the assembly of active ribosomes. Using RNA-based affinity purification, they

show that the IRES RNA is responsible for bringing two initiation factors, eIF3 and eIF2, together on the ribosome. Mutant IRES RNAs become trapped at intermediate stages along the pathway to active 80S ribosomes because one or both of these factors do not associate properly with the smaller (40S) ribosomal subunit.

Although determining the structure and function of these RNA molecules may someday provide therapeutic targets against a number of viruses, Doudna’s interest in them is more basic. “For me, the bigger question is, ‘Can we get enough information so that we can understand the chemical basis for RNA’s many biological functions?’” she says. “It will be exciting to make meaningful comparisons between the chemistry of ribozyme reactions and what happens in protein enzymes that carry out similar reactions.”

If RNA molecules show a breadth of structure and function similar to proteins, the findings may have major implications for one of the most fundamental scientific questions—the origin of life. Scientists have postulated that there might have been an ancient

“RNA world,” where early forms of life were based almost entirely on RNA or an RNA-like molecule. “Obviously, until we build a time machine, we can’t really go back and look at that,” says Doudna, “but what we might be able to do in the laboratory now is to find out, ‘Is it even plausible that RNA could catalyze a variety of different kinds of chemical reactions?’” Doudna continues, “If the answer is yes, then that makes it more likely that the ‘RNA World’ hypothesis might actually be true,” although she says it is a hypothesis that can never truly be tested. Nevertheless, she says, “We hope that we can get information that will help us figure out whether that idea is likely to be true or not.”

Doudna is excited about this possibility and continues to probe the mysteries of RNA in hopes that the results will provide a glimpse of early life on this planet: “I think the idea that RNA might have played a critical role in that process is very tantalizing.”

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