YOUNG SCIENTIST AWARD

Construction of a Minimal, Protein-Free Spliceosome

Saba Valadkhan

Splicing of pre-messenger RNAs to mature transcripts is a crucial step in eukaryotic gene expression. Almost all primary transcripts in higher eukaryotes undergo multiple splicing events, and alternative splicing plays a major role in establishing proteomic diversity in higher eukaryotes. There are numerous examples where splicing and its regulation play key roles in cell growth control, differentiation, and disease. Consistent with its critical role, the spliceosome (the massive ribonucleoprotein particle that catalyzes splicing) has been shown to be the largest and most complicated molecular machine known (*1*).

The complexity of the spliceosome stems not only from its large number of components—almost 300 proteins and five small nuclear RNAs (snRNAs)—but also from the fact that the spliceosome is dynamic, assembling for each splicing event in an elaborate and stepwise fashion involving multiple rearrangements and conformational changes. This complexity poses severe limitations on experimental approaches that can be used to study its function; thus, despite intense research, fundamental aspects of the spliceosome function, such as identity of the catalytic domains and organization of the active site, have remained elusive.

At the start of my graduate research, the existing data pointed to two of the spliceosomal snRNAs, U2 and U6, as the likely catalytic players in the spliceosome. The presence of invariant, functionally crucial domains in these snRNAs, and their similarity to self-splicing group II introns (natural ribozymes that catalyze a reaction identical to the splicing reaction), made them likely candidates for the spliceosomal catalytic domain (*2*). However, despite two decades of experimental effort, neither the catalytic competence of the snRNAs nor direct involvement of other spliceosomal components in catalysis had been established (*3*).

In my graduate work, I addressed this question by trying to build the spliceosomal active site from scratch, by putting together the catalytically essential parts of U2 and U6 and adding short RNA oligonucleotides as splicing substrates. Developing a minimal active site would allow me to test directly the catalytic potential of these two snRNAs in isolation, thereby addressing the question of catalytic domain identity and the possibility of RNA catalysis in the spliceosome. It would also provide a much-needed minimal splicing system for studying the mechanistic aspects of splicing not amenable to study in the context of the whole spliceosome.

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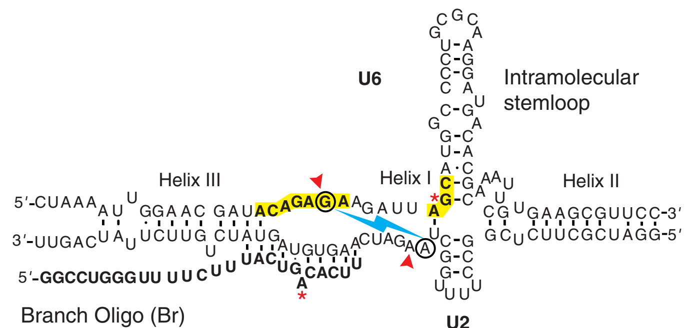
GE Healthcare and *Science*/AAAS are pleased to present the prize-winning essay by Saba Valadkhan, a regional winner from North America, who is the Grand Prize winner of the Young Scientist Award.

To this end, I first showed that U2 and U6 snRNAs have the intrinsic ability to interact with each other in a manner similar to that observed in the active spliceosome. In the absence of any protein factor, U2 and U6 formed a base-paired scaffold that brought the catalytically crucial regions of the two molecules close to each other, and the presence of a functionally important tertiary fold, previously observed in the authentic spliceosome, helped to further position the catalytic domains. Thus, it seemed that the ability to create at least a partial spliceosomal active site was inherent in these two snRNAs (*4*).

Next, we assayed the ability of the U2–U6 complex to recognize and correctly bind the splicing substrates. Two short RNA oligonucleotides, one carrying the consensus sequence for the 5′ splice site (5′SS), the other containing the branch site consensus (Br), were added to the system. Although U2–U6 could efficiently bind Br through base pairing, the 5′SS oligonucleotide, which could form fewer base pairs, was not recognized. Nevertheless, we assayed the system for catalytic activity in the presence of these two oligonucleotides. Interestingly, we obtained an RNA species resulting from a specific catalytic activity (*5*, *6*). Characterization of the RNA, which we named RNA X, showed that its formation depended on intact U2, U6, and Br. However, the 5′SS oligonucleotide was not needed for this reaction. Further characterization of RNA X proved that it resulted from a splicing-related reaction that showed identical sequence and ionic requirements to the authentic splicing reaction. Accordingly, our protein-free reaction and splicing were likely catalyzed by the same, or a very similar, active site. In sum, the protein-free snRNAs were shown to have splicing-related catalytic activity.

The challenge then became finding a way to make the system correctly bind and position the 5′SS, thus setting the conditions for the authentic splicing reaction to occur. It has been shown that, in nematode spliceosomes, certain U6 mutations lead to erroneous use of the 5′ end of U6 in place of the 5′ splice site substrate in splicing reactions (*7*). Since this observation proved it permissible to put the 5′ splice site and U6 on the same molecule, we covalently attached 5′SS to the 5′ end of U6,



The spliceosome active site. The in vitro–assembled, protein-free U2/U6 complex. Yellow highlighted boxes mark the invariant regions in U6, and previously established base-paired regions are indicated. The circled residues connected by thunderbolts can be cross-linked by UV light. Arrows point to residues involved in a genetically proven interaction in yeast (*8*). The base-pairing interactions between Br and U2 snRNA are indicated. The asterisks denote the residues involved in the covalent link between Br and U6 in a splicing-related reaction catalyzed by U2 and U6.

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with a linker sequence joining the two. We engineered a hyperstable hairpin in the linker region to orient 5′SS toward the active site and adjusted the length and sequence of the rest of the linker to help position 5′SS in register with critical active-site nucleotides. Using this chimeric construct, we screened for catalytic products that not only required U2, U6, 5′SS, and Br for their formation but also contained a chemical linkage identical to the product of the first splicing step. One candidate (RNA Y) seemed to satisfy all these requirements. Not only are all the correct sequence elements required for its formation, but our analysis also showed that the chemistry of the reaction was identical to that of the first step of splicing. These studies collectively proved that the spliceosome is an RNA enzyme and a relic from the RNA world.

In addition to providing direct evidence for RNA catalysis in the spliceosome and thus settling the longstanding and central question of identity of the catalytic domain, the minimal system provides a powerful tool for studying the spliceosome. Moreover, the minimal system can be compared with group II introns and the authentic spliceosome in attempts to understand the evolutionary origin of the spliceosome and the transition of the RNA world to the modern, protein-dominated one. Taken together, in addition to putting the spliceosome on the growing list of RNA enzymes playing central roles in cellular function, our results provide an example of the use of designed simple biological systems to address problems not amenable to other approaches.

References and Notes

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2004 Grand Prize Winner

Saba Valadkhan was born and raised in Tehran, Iran. She attended medical school at the Iran University of Medical Sciences from 1989 to 1996 and in 1993 placed fourth in the country in the nationwide Basic Sciences Medical Board Exam. She moved to the United States in 1996 to attend graduate school at Columbia University, New York. There she studied the role of small nuclear RNAs in the human spliceosome under the supervision of Prof. James Manley. While at Columbia University she received awards for both teaching and research. Her thesis was recognized with a Harold Weintraub award from the Fred Hutchinson Cancer Research Center in Seattle. In 2004 she joined Case Western Reserve University in Cleveland, Ohio, as an assistant professor and was named a Searle Scholar the same year.

Regional Winners

North America: Benjamin Tu for his essay, “Deciphering Disulfide Bonds.” Dr. Tu was born in Stanford, California, but grew up in Pennsylvania. After graduating from Harvard in 1998, he moved to the University of California, San Francisco. He was awarded a Howard Hughes Medical Institute Predoctoral Fellowship and joined the laboratory of Dr. Jonathan S. Weissman, where he worked on a long-term problem in protein folding—how disulfide bonds are formed in proteins that traverse the secretory pathway. Dr. Tu obtained his Ph.D. in 2003 and moved to the University of Texas Southwestern Medical Center in Dallas, where he is a postdoctoral fellow in the laboratory of Dr. Steven L. McKnight with a fellowship from the Helen Hay Whitney Foundation. He is currently studying the metabolic cycles of yeast and hopes to apply what he learns to the study of circadian rhythms. In his spare time, he enjoys ultimate frisbee, tennis, ping-pong, puzzles, and Starcraft.

Europe: Christian Haering for his essay, “A Ring for Holding Sister Chromatids Together?” Dr. Haering grew up in Bavaria, Germany. He graduated from the University of Regensburg, Germany, with a diploma in biochemistry in 1999 and in 2000 joined Prof. Kim Nasmyth’s group at the Research Institute of Molecular Pathology (IMP) in Vienna, Austria. Dr. Haering showed that the cohesin complex, required for proper chromosome segregation during cell division, forms a large ring structure with the potential to hold sister chromatids together by trapping them inside rings. His work was recognized with the Austrian Cell Cycle Publication Award in 2002. He received his Ph.D. in 2003 and has recently moved to the Department of Biochemistry at the University of Oxford, UK, where he plans to continue working on the mechanism of the cohesin complex.

Japan: Kunihiko Nishino for his essay, “Analysis of Drug Exporter Gene Libraries Based on Genome Information and Study of Their Regulatory Networks.” Dr. Nishino was born in Kyoto, Japan. After graduating from Kyoto Pharmaceutical University he joined the Graduate School of Pharmaceutical Sciences at Osaka University in 1998. Working in the laboratory of Dr. Akihito Yamaguchi, he developed a postgenomic approach to understanding the regulation and function of xenobiotic exporters. He was awarded a Research Fellowship for Young Scientists from the Japan Society for the Promotion of Science in 2001 and the Kuroya Award from the Japanese Society for Bacteriology in 2002. After receiving his Ph.D. in 2003, he collaborated with Dr. Takeshi Honda at the Research Institute for Microbial Diseases, Osaka University, to extend his knowledge of bacterial pathogenesis. He is now a postdoctoral fellow in the laboratory of Dr. Eduardo A. Groisman at the Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, Missouri.

All Other Countries: Suvendra Bhattacharyya for his essay, “Mitochondrial tRNA Import: Glimpses of a Complex Molecular Machine.” Dr. Bhattacharyya was born in Calcutta, India. He attended the University of Calcutta, where he obtained his B.S. in chemistry in 1996 and M.Sc. in biochemistry in 1998. He then joined the laboratory of Dr. Samit Adhya at the Indian Institute of Chemical Biology (IICB), where he purified the first mitochondrial RNA import complex (RIC) and established a model of tRNA import in *Leishmania* mitochondria. After completing his Ph.D. in 2003, he went to Basel, Switzerland, to join Prof. Witold Filipowicz’s group at the Friedrich Miescher Institute (FMI), of the Novartis Research Foundation, with postdoctoral fellowships from the European Molecular Biology Organisation and the Human Frontier Science Program Organization. At FMI he is engaged in research on microRNA metabolism in mammalian cells. In 2004 he received the Young Scientist Award of the Indian National Science Academy (INSA), India.

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