

Obituary

Christine Guthrie (1945–2022)

Christine Guthrie, a brilliant RNA biologist and beloved mentor, passed away on July 1, 2022, at age 77, after a protracted illness. Among the most influential RNA biologists of her time, Christine and her research group at UCSF made fundamental discoveries that laid the foundation for our understanding of the process by which the spliceosome removes introns from mRNA precursors. Beyond science, she leaves a legacy that underscores the crucial nature of a constructive and collegial environment for fostering the best in trainees and colleagues. Her passing represents a profound loss to the scientific community.

Early years and scientific training at the University of Wisconsin-Madison

Christine Guthrie was born Christine Kampen on April 27, 1945, in Brooklyn, New York, to Irene (Trepel) Kampen and Owen Kampen. After receiving a BS in zoology at the University of Michigan in 1966, she pursued a PhD in genetics at the University of Wisconsin-Madison with the famed ribosome guru Masayasu Nomura. As a graduate student, Christine first used exacting biochemical studies to establish that initiation of bacterial protein synthesis begins on the 30S ribosomal subunit rather than on intact ribosomes. While her 1967 marriage to her high school boyfriend, Stephen Guthrie, did not last, she kept the surname and appeared as Christine Guthrie on her first publication that year.

Christine then turned to genetics to study ribosome subunit assembly. The Nomura lab had shown that, *in vitro*, 30S subunit assembly has a heat-dependent step, so Christine reasoned that a fraction of random cold-sensitive mutations in *E. coli* would be defective in ribosomal subunit assembly. She isolated three such subunit assembly defective (*sad*) mutants. Years later her

lab would use a similar approach to isolate mutations affecting spliceosome assembly and activation. Christine's early experience with both the biochemistry and genetics of a large ribonucleoprotein complex prepared her for later discoveries.

After a brief stint as a visiting scientist at the Max Plank Institute for Molecular Genetics in Berlin, Christine pursued postdoctoral studies on bacteriophage T4 tRNA biosynthesis with Bill McClain, again at UW-Madison. This work was a collaboration with the legendary RNA sequencer Bart Barrell at the MRC LMB in Cambridge, England, and laid the groundwork for the discovery of pre-tRNA processing enzymes. In 1973, at age 28, Christine became a professor of biochemistry and biophysics at UCSF, in a department with only six faculty members, where she served for 47 years. Until 1986, she was the sole female member of the department.

Scientific breakthroughs at UCSF

At UCSF, Christine continued her studies on phage T4-encoded tRNAs. Then, in

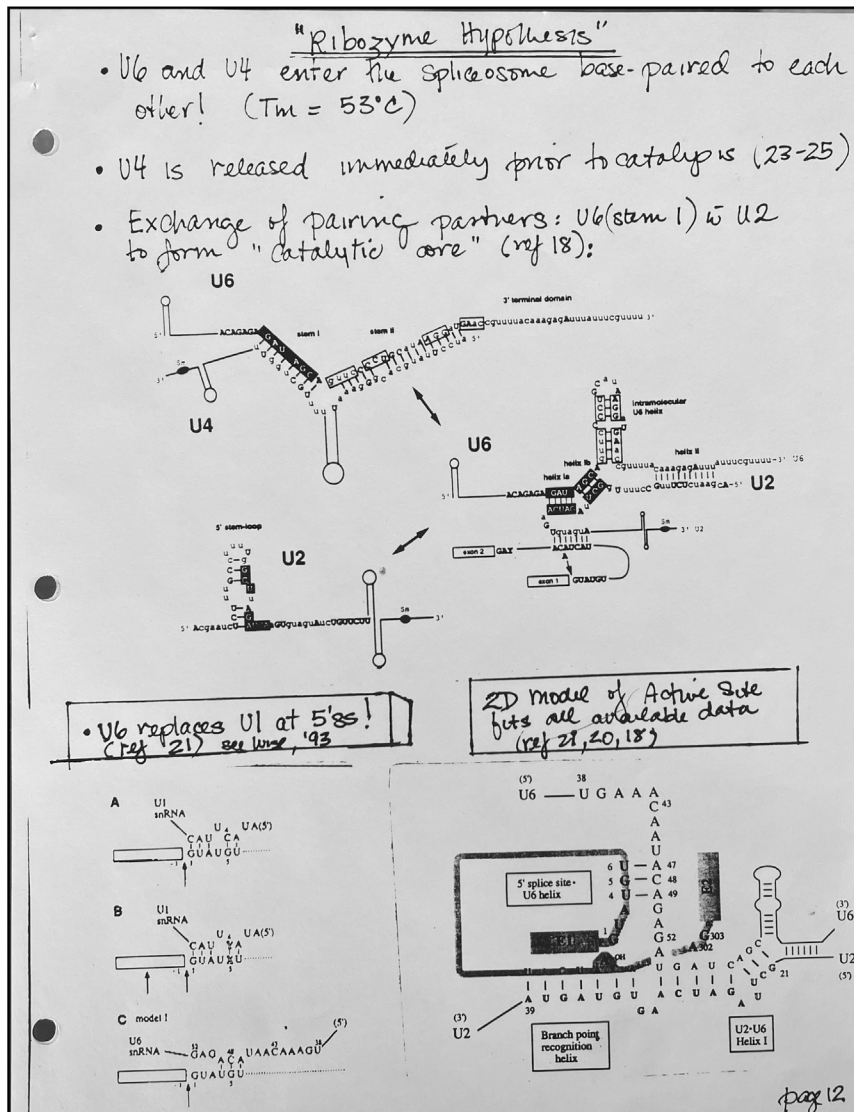


Christine Guthrie (photo courtesy of Jeffery A. Pleiss)

1978, she took the famed yeast course at Cold Spring Harbor Labs taught by Gerry Fink and the late Fred Sherman, after which she turned to studying yeast tRNAs. A few years later, in a career-defining decision, she began to use yeast to study the process of pre-mRNA splicing. In 1980, Joan Steitz, a long-time colleague and friend of Christine's, proposed that snRNPs (small nuclear ribonucleoproteins) are involved in splicing. Christine set out to identify the yeast homologs of the abundant mammalian nuclear snRNAs: U1, U2, U4/U6 (known to be base paired to each other), and U5. Work in HeLa cell extracts had provided evidence for these RNAs having a direct role in splicing. Early momentum in the field was driven by these *in vitro* studies that defined the two-step chemistry of splicing in which sequential transesterification reactions lead to excision of an intron in the form of a lariat-like RNA structure containing an unusual 2'-5' phosphodiester bond and ligation of the flanking exons.

The search for orthologs of the human snRNAs initially hit a brick wall as the yeast snRNAs were not abundant and the first candidates her laboratory cloned turned out to be nonessential small nuclear RNAs (snRNAs), which guide RNA modification. It took several frustrating years to find the yeast homologs of the human snRNAs involved in splicing, but in 1987, the logjam broke and all five revealed themselves within a year. An ongoing collaboration with John Abelson's group at Caltech, initiated more than a decade earlier, provided biochemical confirmation of the presence of the snRNAs in the recently identified yeast spliceosome—a large assembly containing not only snRNAs but also numerous proteins.

It turned out that U1 and U2 were much larger than their human counterparts, while U4 and U5 had limited sequence conservation. U6 was the outlier, being highly conserved and, as in humans,



A page from a handout from a lecture given by Christine Guthrie in the Biological Regulatory Mechanisms course at UCSF, dated February 24, 1994, describing RNA-RNA interactions in the spliceosome

base paired to U4 snRNA, which is how yeast U6 and U4 were identified. Phylogenetic analysis revealed precisely how U4 and U6 pair as evolution provided compensatory base-pairing changes. Unlike the human counterparts, the yeast snRNAs were encoded by single-copy, essential genes, facilitating subsequent genetic studies.

During this time, Christine's lab also initiated studies on intron splice site recognition, using simple intron-containing reporter genes. The first studies

showed that mutating the 5' splice site triggers usage of a nearby cleavage site, but the intermediate produced by this cleavage did not proceed through the second step of splicing. It was later shown by her lab that restoring base-pairing with U1, by then known to pair with the 5' splice site, did not suppress these events. Subsequent work in Christine's lab showed that this was because another snRNA later base paired with the 5' splice site, namely U6.

Exploiting the power of yeast genetics and a compensatory base-pairing approach, Christine's lab defined functions for the snRNAs: U2 base pairs with the sequence in the intron called the branchpoint, the U4/U6 complex is disrupted, enabling U6 to base pair with U2 and form a structure that juxtaposes highly conserved residues with the intron, forming a potential RNA active site. This proposal was based on the prior discovery of group II self-splicing introns, which exhibited splicing chemistry identical to the spliceosome yet could catalyze the reaction without proteins. Thus, in a matter of a few years, Christine's group defined functions for most of the snRNAs and an RNA-RNA interaction network that was dynamic and involved several major rearrangements. The early investment in yeast, while risky, had paid off handsomely. Christine was quickly and widely recognized for these contributions and elected to the US National Academy of Sciences in 1993 at the age of 48.

If the snRNAs are involved in recognition of the intron and, potentially, catalysis of the reaction, then what are the spliceosomal proteins doing? While the RNA-centric studies were progressing, a separate line of investigation in the lab used a classical genetic suppressor selection to identify mutations that restore splicing of an intron with a branchpoint mutation. This selection yielded a novel gene, named *PRP16*, that encodes an ATPase. Subsequent studies on the Prp16 protein led Christine and coworkers to propose that ATP-dependent proofreading clocks in the spliceosome performed functions akin to EF-Tu, the ribosomal GTPase that kinetically proofreads tRNA-mRNA base-pairing. This proposal was met with some skepticism but was ultimately proven to be correct and foundational. Other genetic screens and selections in the lab, often involving cold-sensitive mutants, identified the helicase that disrupts the U4/U6 interaction (Brr2), an activator of Brr2 (a domain of the U5 snRNP protein Prp8), an RNA-binding protein that anneals U4 and U6 snRNAs back together (Prp24), and the ATPase that removes U1 from the 5' splice site

to allow U6 to base pair with it (Prp28). These studies informed the current model for catalytic activation of the spliceosome and inspired award of the 1997 Genetics Society of America Medal to Christine.

In its later years, the Guthrie lab adopted a systems approach to understanding connections between chromatin structure, transcription, pre-mRNA splicing, and nucleocytoplasmic export. These studies included the analysis of the effects of stress responses on splicing, connections between histone modifications and splicing efficiency, and the role of hnRNP (heterogenous nuclear ribonucleoprotein) proteins in orchestrating nuclear processes. In 2011, Christine received the ASBMB-Merck award for outstanding contributions to research in biochemistry and molecular biology. She was one of only two women to win the award in its first 36 years of existence.

While many other labs made important contributions in this area, it is our admittedly biased opinion that none had a greater impact than hers on our understanding of how the spliceosome operates. Extending her legacy, several of Christine's former trainees have made additional key contributions to our understanding of intron recognition, the nature of spliceosomal active site, spliceosomal dynamics, and the role of ATPases in spliceosome disassembly and fidelity.

Because of its dynamic nature, the spliceosome eluded structural biologists for many years. However, advances in cryoelectron microscopy led to high-resolution structures of the rigid core of the spliceosome, beginning in 2015, first in yeast and then in humans. These papers are filled with citations to the work from Christine's lab and from the labs of her trainees. These astonishing structures confirmed the inferences from genetics and biochemistry: the snRNAs form the active site and the ATPases mediate the rearrangements, exactly as Christine had foreseen in the late 1980s and early 1990s. Further validation of the decision to choose yeast to study the process came from structures of human spliceosomes that

revealed that the catalytic core of the spliceosome was essentially identical at the atomic level to that of yeast spliceosomes, a level of structural conservation that few except Christine would have predicted in the 1980s.

Christine the mentor and role model

Aside from being a brilliant scientist, Christine was a revered and beloved mentor, who inspired loyalty and respect from her trainees. She cultivated a supportive lab atmosphere that was simply fun and irreverent with many parties, outings, and summer "RNA camp" trips to a lakeside cabin in northern Idaho. She also had a keen emotional intelligence. Christine could be disarmingly informal and humorous. At other times she could be intimidating (she stood over 6 feet tall) and acerbic and was well known for dispensing "tough love." Like a strong matriarch of a family, Christine commanded respect simply by being who she was.

As a woman in science, Christine endured profound gender discrimination and hostile working environments, particularly during her training years in the late 1960s and early 1970s (detailed in [Guthrie \[2010\]](#)). Nevertheless, she persevered. Christine had a particularly tight bond with many of her female trainees from whom she engendered lifelong adoration by providing a strong, smart, wise, understanding, supportive, yet at times vulnerable, role model. The same was true for many women colleagues and friends.

Christine was open about the challenges of her scientific life. When she was awarded the Lifetime Achievement Award by the RNA Society in 2006 (see editorial in [Nature Structural and Molecular Biology \[2006\]](#)), she spoke about the gender discrimination she had suffered as a young scientist and about her clinical depression and psychiatric hospitalization after a harsh mid-career review and the death of her close colleague Gordon Tomkins in 1975. She also spoke about how a support group of scientists (called simply "Group") arose from this profoundly low point in her life. Group has met since then and was the basis for the book *Every Other Thursday*, written by

her friend and Group cofounder Ellen Daniell. Overcoming these challenges to reach the height of scientific achievement only added to her legendary status. In recognition of her contributions to nurturing the next generation of scientists, Christine was awarded the 1998 Women in Cell Biology Senior Career Recognition Award from the American Society for Cell Biology.

Building the biochemistry and biophysics department and graduate program at UCSF

As one of the first members of the department of biochemistry and biophysics, Christine had an enormous role in building the department to become one of the best in the country, recruiting many faculty who would themselves become leaders in their fields. Christine's input was pivotal in the evaluation of faculty candidates, recruiting them and serving as a valuable mentor to junior faculty. Christine also helped build the first graduate program in molecular and cell biology at UCSF, now called the Tetrad program. Devoted to teaching in the Biological Regulatory Mechanisms course that she designed and started with Keith Yamamoto, Christine never wavered from teaching, lecturing in the course for some 4 decades. Her lecture handouts were sometimes as long as 20 pages, filled with references, "major messages," and hand-written diagrams describing the latest science. During teaching stints, Christine would clear her schedule for weeks to prepare updated lecture materials each year. Christine was an institutionalist and spent her entire faculty career at UCSF building her department and the graduate program.

Christine and the RNA field

It would be an understatement to say Christine was a figure of admiration and respect in the RNA field. Christine had enduring bonds to many central figures and even more junior scientists in the field. She played a role in founding the RNA Society, serving on its Board of Directors from 1991 to 1993 and as its 2000–2001 president. She also served as an associate editor of *RNA*,

the journal published by the RNA Society. Christine had RNA friends across the world. She held these friendships dear, like family. Her closest friend was her husband and collaborator John Abelson, who was with her to the end. Christine Guthrie's enduring curiosity

lives on in the trainees and colleagues she inspired.

ACKNOWLEDGMENTS

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**David A. Brow¹ and
Hiten D. Madhani^{2,*}**

¹Department of Biomolecular Chemistry,
University of Wisconsin School of Medicine
and Public Health, Madison, WI, USA

²Department of Biochemistry and
Biophysics, University of California, San
Francisco, San Francisco, CA, USA

*Correspondence: hitenmadhani@gmail.com

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