Martha R. Stark, Ph.D.

Letter of Application for the RNA Society Outstanding Career Researcher Award

Research Role

My role in the Rader Lab is to manage lab logistics (for example ordering reagents, maintaining equipment, and monitoring spending), train students, and carry out my own research. I focus below on the last of these.

Research Accomplishments

The research in our lab has taken a strange path, from mechanistic studies of pre-mRNA splicing in *S. cerevisiae* to investigating the (d)evolution of introns and splicing in the red alga *C. merolae*. I consider my most important contributions to be the following.

Reimer 2017: In this project, I helped a former undergraduate in the lab investigate the absence of an eighth LSm protein in *C. merolae.* Using AP-MS, I discovered that the cytoplasmic complex responsible for mRNA decapping and degradation, the Pat1 complex, is tightly associated with the splicing machinery, whether the latter is purified via splicing proteins such as Cef1 or the LSm proteins, or via snRNAs. Similarly, purification of Pat1 itself co-purifies most of the 49 splicing proteins present in this organism, particularly those in the U5 snRNP. I believe this to be my most novel discovery to date, in part because it was subsequently shown to hold in human cells, and I am currently attempting to determine the functional significance of this association.

Stark 2015: In the course of investigating *C. merolae* snRNAs, I noticed that we had repeatedly failed to find any candidates for U1. I therefore made a foray into bioinformatics to search for U1 proteins, which should be easier to find than the RNA. While many other splicing proteins were readily identified, not a single U1 protein from any of up to 12 other organisms yielded a convincing hit. This, along with the absence of proteins known to interact with U1 such as Prp28, led us to conclude that the U1 snRNP is indeed missing from this organism, so far the only example of an organism lacking U1.

Hayduk 2012: Working with a talented Master's student, we developed an in vitro reconstitution system for the U4 snRNA. While other snRNAs had previously been depleted from extracts and replaced with exogenous versions that were used to investigate the role of snRNA domains and structures, this had never been accomplished for U4. I observed that since U4 is normally tightly base paired to U6 snRNA, it might be inaccessible to nucleases and therefore refractory to degradation. It had previously been shown, however, that during splicing the U4 particle is released prior to recycling into the U4/U6 complex. This suggested to me that if nuclease-based depletion were carried out under active splicing conditions, U4 might become accessible to nucleases. We were able to demonstrate that this is indeed the case, and used this system to identify a number of functional regions of U4, including some that were previously unknown.

Stark 2006: The ability to ligate RNA into larger molecules provides a route to incorporate site-specific modifications, such as dyes and crosslinkers, and otherwise manipulate the sequence, structure, and chemistry of RNA. Prior to this work, there was only one widely-used method to ligate RNA, and it had highly variable success. Working with Jeff Pleiss, who suggested splinted ligation using RNA ligase instead of DNA ligase, I developed the technique and optimized it to generate high yields with a variety of substrates. This paper has been cited 43 times, suggesting that it has become a useful technique for RNA scientists, and we regularly receive requests for assistance and advice in its use.

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Education

University of California San Francisco, CA, 1992-1997
Ph.D. in Biochemistry

Swarthmore College, PA, 1985–1989
B.A. with Distinction in Biology

Employment History

Research

Adjunct Professor of Chemistry and Research Associate
University of Northern British Columbia 2004-present
with **Dr. Stephen Rader**
•Mechanism of pre-mRNA splicing

Postdoctoral, University of California, San Francisco 1998-2000
with **Dr. Peter Walter**
•Upstream activators of the Unfolded Protein Response pathway: a genetic analysis of signal transduction in a stress response pathway

Graduate, University of California, San Francisco 1992-1997
with **Dr. Alexander Johnson**
•Molecular basis of the DNA-binding specificity of the yeast homeodomain proteins A1 and α2

Research Assistant, University of California, San Francisco 1991-1992
with **Dr. Alexander Johnson**
•Molecular basis of mating type-specific gene repression

Research Assistant, Veterans Administration Hospital, San Francisco 1989-1991
with **Dr. William Seaman**
•Regulation of natural killer cells

Fellowships

Jane Coffin Childs Postdoctoral Fellowship	1998-2000
Howard Hughes Postdoctoral Fellowship	1998

Publications

13. Wong, D.K., Stark, M.R., Rader, S.D., and Fast, N.M. Characterization of Pre-mRNA Splicing and Spliceosomal Machinery in *Porphyridium purpureum* and Evolutionary Implications for Red Algae, *Journal of Eukaryotic Microbiology*, 2021, 68(3). https://doi.org/10.1111/jeu.12844. (PMID: 33569840)

12. Garside, E.L, Whelan, T.A., Stark, M.R., Rader, S.D., Fast, N.M., and A.M. MacMillan. Prp8 in a Reduced Spliceosome Lacks a Conserved Toggle that Correlates with Splicing Complexity across Diverse Taxa, *Journal of Molecular Biology* 2019, 431:2543-2553. (PMID: 31078556)

11. Reimer, K.A., Stark, M.R., Aguilar, L.-C., Stark, S.R., Burke, R.D., Moore, J., Falhman, R.P., Yip, C.K., Kuroiwa, H., Oeffinger, M., and S.D. Rader. The sole LSm complex in *Cyanidioschyzon merolae* associates with pre-mRNA splicing and mRNA degradation factors, *RNA* 2017, 23:952-967.

10. Hudson, A.J., Stark, M.R., Fast, N.M., Russell, A.G., and S.D. Rader. Splicing diversity revealed by reduced spliceosomes in *C. merolae* and other organisms. *RNA Biology*, 2015 DOI: 10.1080/15476286.2015.1094602.

9. Stark, M.R., Dunn, E.A., Dunn, W.S.C., Grisdale, C., Daniele, A., Halstead, M., Fast, N., and S.D. Rader. A dramatically reduced spliceosome in *Cyanidioschyzon merolae*. *Proceedings of the National Academy of Sciences*, 2015, 112:E1191-E1200.

8. Stark, M.R., Hayduk, A.J., and S.D. Rader. In vitro reconstitution of yeast splicing with U4 snRNA reveals multiple roles for the 3′ stem-loop. *RNA*, 2012, 18:1075-1090.

7. Stark MR, Pleiss J, Deras M, Scaringe S, and Rader SD. An RNA ligase-mediated method for the creation of large, synthetic RNAs. *RNA*, 2006, 12:2014-2019.

6. Stark MR, Escher D, Johnson AD. A trans-acting peptide activates the yeast a1 repressor by raising its DNA-binding affinity. *EMBO J*. 1999 Mar 15;18(6):1621-9.

5. Redd MJ, Stark MR, Johnson AD. Accessibility of alpha 2-repressed promoters to the activator Gal4. *Mol Cell Biol*. 1996 Jun;16(6):2865-9.

4. Li T, Stark MR, Johnson AD, Wolberger C. Crystal structure of the MATa1/MAT alpha 2 homeodomain heterodimer bound to DNA. *Science*. 1995 Oct 13;270(5234):262-9. Erratum in: *Science* 1995 Nov 17;270(5239):1105.

3. Stark MR, Johnson AD. Interaction between two homeodomain proteins is specified by a short C-terminal tail. *Nature*. 1994 Sep 29;371(6496):429-32. Erratum in: *Nature* 1994 Nov 17;372(6503):279.

2. Phillips CL, Stark MR, Johnson AD, Dahlquist FW. Heterodimerization of the yeast homeodomain transcriptional regulators alpha 2 and a1 induces an interfacial helix in alpha 2. *Biochemistry*. 1994 Aug 9;33(31):9294-302.

1. Seaman WE, Niemi EC, Stark MR, Goldfien RD, Pollock AS, Imboden JB. Molecular cloning of gp42, a cell-surface molecule that is selectively induced on rat natural killer cells by interleukin 2: glycolipid membrane anchoring and capacity for transmembrane signaling. *J Exp Med*. 1991 Jan 1;173(1):251-60.