JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 8, NO. 9, 2023

EDITORIAL COMMENT

Reading a Good Transcript Soothes MYZAPed Heart*

Emma L. Robinson, PhD,[a,b] Timothy A. McKinsey, PhD[a,b]

Post-transcriptional chemical modifications of RNA, referred to collectively as epitranscriptomic marks, can dramatically impact mRNA stability, localization, and translation. As with the epigenome, regulation of the epitranscriptome involves the actions of "writer," "eraser," and "reader" proteins, which add, remove, and associate with the chemical marks, respectively.

In recent years, much attention has focused on the roles of N^6-methyladenosine (m6A) modification of RNA in the control of cardiac homeostasis and disease. m6A methylation is governed, in part, by the methylase methyltransferase-like 3 (METTL3) enzyme, which places the methyl group on exons or 3' or 5' untranslated regions of mRNA. Prior work by the Accornero group demonstrated that m6A modification of mRNA is elevated in response to hypertrophic stress in the heart, and is enriched in transcripts that encode pro-growth signal transduction mediators.[1] Transgenic overexpression of METTL3 in mouse cardiomyocytes stimulated physiological growth of the heart, whereas cardiomyocyte-specific deletion of the enzyme led to spontaneous heart failure, illustrating a requirement for m6A methylation of RNA in the maintenance of cardiac homeostasis.

Seemingly paradoxically, other studies suggested that reducing m6A methylation in the heart is beneficial because cardiac overexpression of the m6A eraser, FTO, is protective in mouse models of myocardial infarction, whereas cardiomyocyte-specific knockout of this enzyme exacerbates pressure overload-induced cardiac remodeling.[2,3] Although it is initially counterintuitive that decreasing m6A methylation, either through METTL3 deletion or FTO overexpression, elicits both deleterious and salutary effects in the heart, there is precedent for this type of complexity from work on epigenetics, where reducing or increasing histone acetylation by inhibiting acetyltransferases (writers) or deacetylases (erasers), respectively, both appear to block adverse cardiac remodeling. Together, the findings highlight the importance of "epi-writers" and "epi-erasers" in the heart, but also underscore the need for additional mechanistic studies that define the biochemical and functional outcomes of altering chemical marks on specific writer/eraser mRNA and protein substrates.

In this issue of *JACC: Basic to Translational Science*, Golubeva et al[4] addressed the role of the m6A reader protein, YTHDF2, in the heart. Just 1 month after cardiomyocyte-specific deletion of the gene encoding YTHDF2, using the *α-myosin heavy chain MerCreMer* driver, mice developed systolic dysfunction and lung congestion, and exhibited multiple cellular and molecular hallmarks of heart failure, including eccentric cardiomyocyte hypertrophy, cardiac fibrosis, and pathologic stress-associated gene expression. Unbiased proteomics from control or YTHDF2 conditional knockout (cKO) cardiomyocytes revealed substantial remodeling of the proteome in the absence of the reader protein, with 81 up-regulated and 122 down-regulated proteins in cKO cardiomyocytes. Follow-up experiments focused on the 5 most up-regulated proteins, and RNA immunoprecipitation studies most convincingly revealed the mRNA encoding Myocardial Zonula Adherens Protein (MYZAP) as an m6A-modified target for YTHDF2 binding.

*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

From the [a]Department of Medicine, Division of Cardiology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA; and the [b]Consortium for Fibrosis Research & Translation, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2023.05.002

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 8, NO. 9, 2023
SEPTEMBER 2023:1195–1197

MYZAP was originally described by the Frey group as an intercalated disc (ID)-enriched protein that transmits signals to the cardiomyocyte nucleus to alter gene expression via the Rho/SRF axis.[5] Either gain- or loss-of-function of MYZAP in the mouse heart results in pathologic cardiac remodeling, and in humans, mutations in the gene encoding MYZAP have been linked with severe dilated cardiomyopathy and atrial fibrillation, further illustrating the crucial importance of this YTHDF2 target in controlling homeostasis of the heart.

Using a cell-based model, Golubeva et al[4] provide evidence to suggest that YTHDF2 deletion reduces decay of *Myzap* mRNA, thereby resulting in enhanced MYZAP protein production. Furthermore, the authors demonstrate that YTHDF2, but not the related family members YTHDF1 and YTHDF3, localizes to the IDs of cardiomyocytes. These findings suggest the intriguing possibility that YTHDF2 provides a regulatory mechanism to guarantee proper, localized production of proteins that comprise the ID, a mechanism that would likely complement compartmentalized protein translation/degradation processes that govern ID maintenance.[6] Consistent with this possibility, 16% of YTHDF2 cKO mice developed ventricular arrhythmias, whereas none of the control mice displayed cardiac electrical abnormalities, suggesting that YTHDF2 reader activity is required for correct formation of IDs, which ensures appropriate conduction of electrical impulses between cardiomyocytes.

Golubeva et al[4] performed elegant and well-controlled experiments and presented data that are convincing and not overinterpreted. The current findings will undoubtedly serve as a springboard for future investigation that further advances our understanding of the roles of epitranscriptomic marks in the control of cardiac homeostasis and disease. Several issues remain to be resolved. For example, what is the constellation of m6A-methylated mRNAs that are read by YTHDF2 in the heart? As pointed out by the authors, many of the changes in protein expression observed in YTHDF2 cKO hearts are likely due to indirect, secondary effects elicited in response to cardiac remodeling events. Unbiased, transcriptome-wide analyses designed to sequence YTHDF2-bound RNAs that are also m6A methylated should help resolve this issue and will also assist with determining the extent to which MYZAP up-regulation contributes to pathogenesis in cKO hearts. Additionally, to address this latter point, a “rescue” experiment could be performed by crossing YTHDF2 cKO mice with MYZAP heterozygous knockout mice.

The authors used an H9C2 cell-based assay to demonstrate that small interfering RNA knockdown of YTHDF2 reduces *Myzap* mRNA decay. However, total *Myzap* mRNA abundance was not significantly elevated in YTHDF2 cKO hearts. Thus, it is possible that YTHDF2 regulates MYZAP protein expression through independent mechanisms, such as by controlling translation of its transcript. Alternatively, the apparent lack of an effect of YTHDF2 cKO on the level of total *Myzap* mRNA may reflect the fact that the reader protein only controls *Myzap* transcripts that are in close proximity to the ID, and thus analysis of total RNA could be masking effects of cKO on this localized pool of RNA. Expanded efforts to better understand spaciotemporal effects of YTHDF2-mediated m6A reading on post-transcriptional regulation of *Myzap* mRNA are warranted. This could possibly be achieved by using single-molecule mRNA fluorescence in situ hybridization to analyze the dynamics of *Myzap* transcript regulation in intact cells.[6]

How is YTHDF2 regulated in the heart? The authors convincingly show that YTHDF2 protein expression is elevated in hearts of mice with pressure overload-induced heart failure. It will be interesting to determine if up-regulation of this m6A reader protein is a generalizable effect that occurs in multiple forms of heart failure, regardless of etiology, and if YTHD2 induction contributes to the pathogenesis of human cardiac disease. If so, it will be important to address the mechanism(s) that control YTHDF2 expression and function, for example, to determine if the gene encoding the m6A reader is transcriptionally induced in response to cardiac stress signals, whether its mRNA is subject to post-transcriptional control, and if YTHDF2 protein reader activity and/or localization are altered by post-translational modifications. Additionally, the potential interplay between YTHDF2 and other epitranscriptomic regulators should be considered. For example, in the METTL3 cKO mouse model, is YTHDF2 association with *Myzap* mRNA reduced, leading to increased MYZAP protein expression and deranged ID formation with resultant pathologic remodeling of the heart?

What is the therapeutic potential of pharmacologically manipulating YTHDF2 for the treatment of heart failure? At first glance, the findings of Golubeva et al[4] suggest that YTHDF2 is not a viable target for the treatment of heart disease because deleting this m6A reader in cardiomyocytes triggers spontaneous heart failure. However, deleting an “epi regulator” does not always phenocopy its inhibition, as illustrated by studies of the BRD4 acetyl-histone reader protein. Indeed, deletion of BRD4 from mouse cardiomyocytes leads to severe adverse remodeling of the heart, whereas small molecule inhibitors such as

JQ1, which disrupt binding of BRD4 to acetylhistones, potently suppress pathologic hypertrophy and fibrosis, and improve contractile function of the heart. YTHDF inhibitors that prevent binding of the readers to m6A-methylated RNA are emerging and should eventually be assessed for efficacy in heart failure models.

This important work by Golubeva et al[4] has opened up a completely new avenue of study in the molecular cardiology field. Results of forthcoming studies will unquestionably enhance our understanding of the molecular foundations of normal heart function, and may unveil approaches by which readers of mRNA transcripts can be "drugged" to soothe a sapped heart.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

Dr Robinson was funded by the American Heart Association (Grant 829504). Dr McKinsey received funding from National Institute of Health through grants HL116848, HL147558, DK119594, HL127240, and HL150225. Dr McKinsey is on the scientific advisory boards of Artemes Bio and Eikonizo Therapeutics and has a subcontract from Eikonizo Therapeutics related to an Small Business Innovation Research grant from the National Institutes of Health (HL154959).

ADDRESS FOR CORRESPONDENCE: Dr Timothy A. McKinsey, Department of Medicine, Division of Cardiology, University of Colorado Anschutz Medical Campus, Aurora, Colorado 80045-2507, USA. E-mail: timothy.mckinsey@cuanschutz.edu.

REFERENCES

1. Dorn LE, Lasman L, Chen J, et al. The N(6)-methyladenosine mRNA methylase METTL3 controls cardiac homeostasis and hypertrophy. *Circulation*. 2019;139:533–545.

2. Berulava T, Buchholz E, Elerdashvili V, et al. Changes in m6A RNA methylation contribute to heart failure progression by modulating translation. *Eur J Heart Fail*. 2020;22:54–66.

3. Mathiyalagan P, Adamiak M, Mayourian J, et al. FTO-dependent N(6)-methyladenosine regulates cardiac function during remodeling and repair. *Circulation*. 2019;139:518–532.

4. Golubeva VA, Dorn LE, Gilbert CJ, et al. Loss of YTHDF2 alters the expression of m^6A-modified Myzap and causes adverse cardiac remodeling. *J Am Coll Cardiol Basic Trans Science*. 2023;8:1180–1194.

5. Seeger TS, Frank D, Rohr C, et al. Myozap, a novel intercalated disc protein, activates serum response factor-dependent signaling and is required to maintain cardiac function in vivo. *Circ Res*. 2010;106:880–890.

6. Lewis YE, Moskovitz A, Mutlak M, Heineke J, Caspi LH, Kehat I. Localization of transcripts, translation, and degradation for spatiotemporal sarcomere maintenance. *J Mol Cell Cardiol*. 2018;116:16–28.

KEY WORDS epitranscriptomics, heart failure, intercalated disc, m6A methylation, methyl-RNA reader protein, Myzap, RNA methylation