JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 9, NO. 6, 2024

EDITORIAL COMMENT

Turn(over) the Page

Advancing Understanding of Proteome Dynamics After Heart Attack*

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Maintenance of cellular protein quality through constant turnover of the proteome is essential for cellular viability and longevity. In cardiomyocytes, protein quality control is particularly vital as most of these cells are as old as the organism itself, are extremely metabolically active, and contract persistently, which leads to oxidative stress and protein denaturation.[1] Given the reliance of cardiomyocytes on effective protein turnover, it is unsurprising that failure at any phase in this process (synthesis, degradation, or both) disrupts cardiomyocyte protein homeostasis and can lead to contractile dysfunction or even cell death. Indeed, many studies have identified alterations in cardiomyocyte protein quality control in disease settings, which has led to interest in targeting cardiac protein turnover as a possible therapeutic strategy. However, although the importance of cardiomyocyte protein turnover for maintaining optimal contractile function has long been appreciated, how this process is altered at early stages of heart disease onset remains poorly understood.

In this issue of *JACC: Basic to Translational Science*, Liu et al[2] examined cardiomyocyte proteome dynamics in vivo in mice at baseline and in a model of acute heart failure resulting from myocardial infarction (MI). To monitor proteome turnover solely in cardiomyocytes, the authors devised an elegant bioorthogonal approach using mice expressing the L274G mutant methionyl-tRNA synthetase (MetRS)[3] under an inducible, cardiomyocyte-specific promoter. The mutant MetRS (MetRS*) has an expanded amino acid binding site, which enables exogenous labeling of newly synthesized proteins by delivery of a non-canonical amino acid and methionine analogue, azidonorleucine (ANL). Importantly, ANL has an active azide moiety, which Liu et al used for alkyne click chemistry labeling of newly synthesized proteins. This is the first example of the MetRS* mouse being used for a focused cardiac study, although the method has been previously used in vitro and in other organ systems in vivo.[4] The compatibility of click chemistry with immunofluorescence microscopy, which the authors[2] exploited to identify subcellular localization of newly synthesized proteins, allows for certain advantages over other common in vivo nascent protein labeling approaches (eg, stable isotope labeling with deuterium oxide or heavy amino acids), where labels are not so readily compatible with high-resolution imaging.

After inducing MetRS* expression in adult mice, Liu et al[2] delivered a single bolus of ANL and harvested hearts 1 to 5 days later to examine incorporation of ANL into newly synthesized proteins. Biotin labeling of ANL-containing proteins via click chemistry revealed robust incorporation of this amino acid at 24 hours' postinjection. ANL expression in protein lysates then steadily declined until it was barely detectable 4 days later, supporting the conclusion that turnover of most proteins in cardiomyocytes occurs rapidly. Indeed, the average protein half-life in cardiomyocytes was estimated to be just 2 days. These findings were further confirmed by using a complementary microscopy approach on tissue sections in which the ANL was labeled with a fluorescent tag, TAMRA alkyne (Sigma-Aldrich). These imaging

*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.

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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.2024.05.002

experiments also identified the subcellular localization of the newly synthesized proteins, which the authors found were enriched at the intercalated disc and nuclei of cardiomyocytes, suggesting that proteins in these structures are especially dynamic.

After this initial characterization, the authors[2] next used immunoprecipitation of biotin-labeled ANL proteins followed by mass spectrometry to identify newly synthesized proteins 1 day after injection of ANL. This approach revealed that the most abundant newly synthesized proteins at baseline in cardiomyocytes are those involved in mitochondrial metabolism and contractility. The finding that these proteins were the most abundant is perhaps not surprising, as they are also among the most highly expressed in cardiomyocytes, which are densely packed with myofilaments and mitochondria. However, the data further support that these proteins are also highly dynamic and rapidly turned over. For example, sarcomeric actin, one of the most abundant proteins in cardiomyocytes, was found to have a half-life of approximately 2 days.

Having established the baseline state, the investigators[2] next turned their attention to the disease model to examine how protein synthesis is affected by MI. Using detailed microscopy analyses of cardiac tissue sections fluorescently labeled by click chemistry, the authors found that ANL intensity decreased in the infarct border zone, suggesting a reduction in newly synthesized proteins in cardiomyocytes in this region. Importantly, this was not due to poor perfusion of injected ANL, as follow-up experiments with ANL delivered 6 hours before coronary artery ligation led to the same result. Although the authors did not attempt to specifically characterize the newly synthesized proteins in the infarct border zone compared with remote regions, they did assess the global left ventricular proteome by using mass spectrometry. Proteins that increased in expression after MI included those involved in mitogen-activated protein kinase signaling and lipid metabolism, whereas decreased expression was found for mitochondrial metabolism and glycolysis proteins. Although the infarct border zone was not specifically measured, this finding suggests that proteins decreased in the region likely included metabolic factors.

Possibly the most interesting finding of the study by Liu et al[2] was discovered when the authors tested whether proteome changes post-MI are recapitulated at the messenger RNA level. They used published single-nucleus RNA sequencing and spatial transcriptomics data and annotated the remote and border zones of ischemia using validated marker genes of these regions to obtain spatial information on the genes encoding their own identified MI-regulated proteins. Notably, the spatial transcriptomics analysis identified increased messenger RNA expression in the border and ischemic zones for the proteins that were decreased post-MI, which suggests there is an uncoupling of transcription and translation in these cells after ischemic injury. One might speculate that this is due to reduced mitochondrial aerobic respiration in the hypoxic cardiomyocytes, and consequently decreased adenosine triphosphate levels, which leads to insufficient cellular energy to maintain protein synthesis despite an abundance of mature transcripts.

The study by Liu et al[2] shows that MetRS* mice are a useful tool for characterizing cardiomyocyte proteome dynamics in vivo, and it represents a seminal advancement in the areas of cardiac proteome labeling and protein turnover. Particularly valuable is the ability to label newly synthesized proteins in a single cell type, rather than labeling proteins in all cardiac cell types, as is the case with stable isotope labeling by deuterium oxide or heavy amino acids. Such bulk labeling approaches cloud interpretations for half-lives of proteins that are found in multiple cell types, which is most proteins. Possibly, it is this feature that led to the average cardiomyocyte protein half-life being approximately 2 to 3 days in this study, although previous examinations using stable isotope labeling have identified protein half-lives in the heart that are 2 to 4 times higher.[5] However, it is interesting that even half-lives of many sarcomere proteins were 2 to 3 days in this study[2] (much shorter than previous estimations) as these proteins are unique to cardiomyocytes and would thus not be affected by methods analyzing bulk heavy isotope-labeled homogenates from the whole left ventricle. Future head-to-head comparisons of ANL vs. stable isotope labels in MetRS* mice will be a valuable next step to help fully interpret and possibly reconcile these differences.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

Dr Leinwand acknowledges support from the National Institutes of Health (R01GM029090). Dr Martin acknowledges support from the National Institutes of Health (F32HL170637). Dr Leinwand is a cofounder of MyoKardia, acquired by Bristol Myers Squibb. MyoKardia and Bristol Myers Squibb were not involved in this study. Dr Martin has reported that he has no relationships relevant to the contents of this paper to disclose.

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JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 9, NO. 6, 2024
JUNE 2024:808–810

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KEY WORDS mass spectrometer, methionyl-tRNA synthetase, myocardial infarction, newly synthesized protein