

Seeing the Forest for the Trees

Brian O'Rourke, Ting Liu, D. Brian Foster

Although tens, or even hundreds, of molecular or pharmacological interventions have been shown to affect the course of heart failure in animal models, few have translated to clinical therapies. This likely stems from the fact that biological robustness and flexibility are conferred by the evolution of redundant or adaptive signaling and gene expression pathways, which lead to not one, but multiple “master” control nodes in the network, making it difficult to pinpoint the most effective single target intervention. Systems biology tools and approaches are helping to define how the heart is remodeled during stress and to define important control points, even as they further highlight the scope of our ignorance. Applying these analyses to time-dependent changes in heart failure is essential to understand critical thresholds for aberrant nonlinear cellular responses that lead to emergent events, such as sudden cardiac death.

Trying to understand the mechanisms behind heart failure often brings to mind the analogy of science as “a hungry furnace that must be fed logs from the forests of ignorance that surrounds us. In the process, the clearing we call knowledge expands, but the more it expands, the longer its perimeter and the more ignorance comes into view.”¹ Extending this further, we scientists often act as independent woodcutters in our own little clearings, either focusing our reductionist minds on taking the tree down like a leafcutter ant rather than by chainsaw, or attempting to frame our favorite target as the master key that unlocks the secret to curing the disease (a must to enchant those study section reviewers). From the literature, it seems that there are many such master keys that prevent or reverse cardiac dysfunction related to heart failure (HF) in animal models—fixing various defects in SR Ca²⁺ uptake, ryanodine receptor leak, repolarization reserve, oxidative stress, nitric oxide, cGMP signaling, β -receptor desensitization, CaM kinase, anaplerosis, oxidative phosphorylation, signaling through TGF- β , MAP kinase, GSK3- β , etc, all have been shown to be effective in one animal model or another. So it seems that heart failure should be cured by now. Unfortunately, the best medical treatment still only offers a delay in contractile failure, extension of the time to morbidity

and mortality, and a backstop against sudden death by means of implantable defibrillators. No doubt, these advances have made a major impact, yet individual outcomes are still highly unpredictable.

Systems biology approaches attempt to harness the efforts of all of the lumberjacks to fell those resistant stands of forest between the clearings. This has to occur at multiple levels. Elucidating how the subsystems of the cell (eg, ions, energy, contraction, signal transduction, and gene/protein expression/modification) interact must fit in to the larger picture of the heart as a syncytium with myocyte and nonmyocyte components, and at a still larger scale, how the cardiovascular system is regulated by neural, hormonal, metabolic, immune, and volume regulatory elements of the organism. Predicting emergent phenomena, such as sudden death, requires a better understanding of the system from top to bottom. Significant challenges to this approach exist, including (1) the large number of free parameters to deal with and a poor understanding of their interactions, (2) dynamic remodeling of the system as the pathophysiology progresses, and (3) acute events/stresses that occur locally that may or may not scale to the cell/organ/organism level.

Harnessing technological developments to tease apart the system has contributed in a deep way to understanding the multifactorial nature of heart failure, yet interpretation is often clouded by nature's robust and flexible design philosophy. Knockout of one gene, or inhibition of one pathway, does not necessarily illuminate its role in the physiological or pathological process owing to redundancy and adaptation. Redundancy in signal transduction² and gene expression³ is well documented and can occur through the activation of paralogous pathways or recruitment of different components performing similar or overlapping tasks to achieve functional plasticity, referred to as degeneracy.⁴ Adaptive plasticity also explains why knockout of processes assumed to be vital, such as the cardiac sarcolemmal Na⁺/Ca²⁺ exchanger⁵ or the mitochondrial Ca²⁺ uniporter,⁶ often shows a mild phenotype, or one that is revealed only under stress. In such cases, the question arises as to whether one is learning about the normal role of the targeted protein or the behavior of the system in its adapted state. Unfortunately, essential information to enable assessment of the alterations in the transcriptome, proteome, or metabolome of the adapted state is rarely provided if the main hypothesis of the study was more or less supported, sometimes requiring impressive feats of logical contortion. This problem will not diminish as it becomes easier and less expensive to make global mutations through new technologies, such as CRISPR/Cas9, to knockout genes or mutate post-translational modification sites. Conditional or inducible genetic manipulations partially mitigate these concerns, decreasing the chances of long-term adaptation, but do not eliminate short-term workarounds through adaptive

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

From the Division of Cardiology, Department of Medicine, The Johns Hopkins University, Baltimore, MD.

Correspondence to Dr Brian O'Rourke, Division of Cardiology, Department of Medicine, The Johns Hopkins University, 720 Rutland Ave, 1060 Ross Bldg, Baltimore, MD 21205-2195. E-mail bor@jhmi.edu (*Circ Res.* 2016;119:1170-1172.

DOI: 10.1161/CIRCRESAHA.116.310066.)

© 2016 American Heart Association, Inc.

Circulation Research is available at <http://circres.ahajournals.org>

DOI: 10.1161/CIRCRESAHA.116.310066

post-translational or epigenetic mechanisms. In all cases, the key is to apply available technologies to get a sense of how the baseline is changed to better evaluate the impact of a gene on the system before applying the disease-inducing stimulus (pressure-overload, ischemia-reperfusion injury, etc). Does the gene knockout increase expression of a different isoform? Is there evidence of a hormetic response? Is the effect on the heart secondary to the impact of the genetic mutation on a different organ (liver, pancreas, kidney, and brain)? These types of questions are typically pursued only if the results cannot be shoehorned into the presumed model or if no obvious phenotype is observed—we would argue that they are no less important when the results fit neatly into the proposed hypothesis.

In this light, omics technologies are rapidly advancing to a point where system-wide changes, induced by genetic engineering or disease induction, can be assessed fairly comprehensively. Coupled with advanced bioinformatics, strong inferences can be made about causality in terms of pathways and transcription factors active during adaptive or pathophysiological responses. We have recently applied a triple-omic strategy (transcriptome, proteome, and metabolomic) to investigate the ventricular adaptations during the compensatory hypertrophy and failure in a guinea pig HF/SCD model.⁷ This nonischemic model of HF displays acquired long QT, decreased repolarizing K⁺ currents, impaired Ca²⁺ handling, and spontaneous arrhythmogenic sudden death, providing the opportunity to study how time-dependent cardiac remodeling might contribute to a lowered threshold for acute events to bring about fatal consequences.⁸ We learned several lessons from this approach and also recognized important limitations. First, as others have previously reported,⁹ there was a significant degree of discordance between the transcriptome and proteome. Hypertrophic growth was accompanied by increased transcripts of fibrosis-inducing and acute-phase immune response pathways (FGF, TGFβ, and TNFα), although few quantitative changes in the proteome were observed in the compensated state. However, further degradation of contractile function to heart failure was accompanied by strong downregulation of metabolic, antioxidant defense, and hypertrophic growth pathways, most convincingly revealed by network analysis of the proteomic data. Specific deficiencies in enzymes involved in fatty acid activation, transport, and oxidation were consistent with changes observed in the metabolome, which also revealed the accumulation of an unexpected, potentially toxic, metabolite (2-methylcitrate). The overall picture pointed to vulnerability of mitochondrial energy supply and redox balance, consistent with functional observations in failing cardiac myocytes.⁸ Each methodology provided a unique window into the process of cardiac decompensation, which was distinct from the hypertrophic growth phase, and each had its own limitations. The mature technology of transcriptome analysis had the highest coverage of expressed genes but did not necessarily predict the protein profile, while proteomics provided a direct assessment of the actual functional molecules in the cell, but with incomplete coverage of low abundance or membrane-localized proteins. Moreover, a true system-wide analysis requires the quantification of all proteoforms¹⁰ generated by the many post-translational modification mechanisms, localization of each proteoform, and

determination of its binding partners (interactome).¹¹ Thus far, this has only been pursued for a few selected modifications and protein complexes. Metabolomics gives a readout of changes in the intracellular milieu but presents the challenge of trying to infer enzyme fluxes from pathway intermediates (unless tracer methods are used) and could benefit from further improvements in coverage and sensitivity.

A fully integrated and complete omic analysis incorporating all available technologies is unrealistic in most cases. Nevertheless, important insights can be gained from limited data sets by coupling the output with bioinformatics tools that harness cumulative knowledge bases to identify pathways and factors underlying system remodeling. Keeping in mind the caveats mentioned above about single genes or master keys, statistical methods such as causal network analysis¹² can be used to build hierarchies of potential control nodes in the cellular protein expression network. In our case, the transition from compensated hypertrophy to heart failure clearly pointed to defects in PGC1α, retinoic acid, and mitochondrial transcription/replication pathways. At some point, the nuclear integration of cytoplasmic second messengers (eg, Ca, ROS, cyclic nucleotides, and nitric oxide) or other signal transducers fails to maintain the machinery required for mass–energy transformation. We think that this results in a threshold-lowering effect for disruptive emergent events. For example, a decline in metabo–redox coupling, that is, a decrease in the ability of the heart to provide reducing equivalents for ATP production (NADH) and ROS scavenging (NADPH) at sufficient rates to meet metabolic/oxidative demand, lowers the threshold for nonlinear positive feedback loops to occur, resulting in cell-wide ROS overload, collapse of the mitochondrial membrane potential, uncontrolled Ca²⁺ release, and arrhythmias. This combination of flux imbalances under stress, and vulnerability of the myocardial substrate accounts for an increase in the probability of a seemingly unpredictable event, such as cell death, spontaneous Ca²⁺ release, or a cellular arrhythmia to scale to the level of the whole heart.

The bridge between altered cytoplasmic signals and nuclear metabo–redox gene expression, and how it might fail, is still unknown. However, based on the previous work on transcriptional control of PGC1α, as well as a high representation of cAMP response elements and/or PPARγ transcriptional elements in the promoter regions of the downregulated proteins, we propose that failed CREB/CREB-binding protein transcriptional signaling could be a factor.⁷ Of course, given the redundancy argument, it is likely that CREB would not be the only nuclear integrator regulating metabolic and antioxidant pathways. For example, the proteomic analysis also revealed that retinoic acid and estrogen receptor signaling are also impaired in the HF/SCD model, and there is substantial target overlap in these pathways, particularly with respect to lipid transport, β-oxidation, and ion transport. Indeed, it has been elegantly argued that the accumulation of multiple master regulators for critical cellular processes occurs naturally during evolution via the accrual of neutral (nonadaptive) gains of regulatory connections.³ If that is the case, we should probably give up on the notion of a single master key to heart failure; instead, we should define the groups of master

regulators that positively impact outcomes and figure out how their inputs or interconnections fail. Much additional work needs to be done to confirm these hypotheses and conceive of ways to correct possible defects for therapeutic benefit. In particular, it will be important to apply available omics technologies to better define how the system is changing in time, and how functional perturbations are handled as the heart chronically remodels subsequent to load-, oxidative-, or ischemia-induced stress.

In summary, we must remain vigilant to avoid falling into the trap of single target cures for heart failure. Nevertheless, detailed investigation of the control properties of the system may indeed identify those processes that could be targeted effectively without being circumvented by pathophysiological redundancy or causing further maladaptation. The concept of a variable emergence threshold as chronic proteome remodeling occurs is worthy of further investigation to explain the increased propensity for arrhythmias, increased rates of cell dropout, or sudden death associated with heart failure.

Sources of Funding

This study was supported by National Institutes of Health P01HL077180, R01HL101235, and Johns Hopkins University Proteomics Innovation Center in Heart Failure.

Disclosures

None.

References

1. Ridley M. *Genome: The Autobiography of a Species in 23 Chapters*. New York, NY: HarperCollins; 1999.
2. Sun C, Bernards R. Feedback and redundancy in receptor tyrosine kinase signaling: relevance to cancer therapies. *Trends Biochem Sci*. 2014;39:465–474. doi: 10.1016/j.tibs.2014.08.010.

3. Sorrells TR, Johnson AD. Making sense of transcription networks. *Cell*. 2015;161:714–723. doi: 10.1016/j.cell.2015.04.014.
4. Maleszka R, Mason PH, Barron AB. Epigenomics and the concept of degeneracy in biological systems. *Brief Funct Genomics*. 2014;13:191–202. doi: 10.1093/bfgp/elt050.
5. Henderson SA, Goldhaber JI, So JM, Han T, Motter C, Ngo A, Chantawansri C, Ritter MR, Friedlander M, Nicoll DA, Frank JS, Jordan MC, Roos KP, Ross RS, Philipson KD. Functional adult myocardium in the absence of Na⁺-Ca²⁺ exchange: cardiac-specific knockout of NCX1. *Circ Res*. 2004;95:604–611. doi: 10.1161/01.RES.0000142316.08250.68.
6. Pan X, Liu J, Nguyen T, Liu C, Sun J, Teng Y, Fergusson MM, Rovira II, Allen M, Springer DA, Aponte AM, Gucek M, Balaban RS, Murphy E, Finkel T. The physiological role of mitochondrial calcium revealed by mice lacking the mitochondrial calcium uniporter. *Nat Cell Biol*. 2013;15:1464–1472. doi: 10.1038/ncb2868.
7. Foster DB, Liu T, Kammers K, O'Meally N, Yang N, Papanicolaou KN, Talbot CC Jr, Cole RN, O'Rourke B. Integrated omic analysis of a Guinea pig model of heart failure and sudden cardiac death. *J Proteome Res*. 2016;15:3009–3028. doi: 10.1021/acs.jproteome.6b00149.
8. Liu T, Takimoto E, Dimaano VL, DeMazumder D, Kettlewell S, Smith G, Sidor A, Abraham TP, O'Rourke B. Inhibiting mitochondrial Na⁺/Ca²⁺ exchange prevents sudden death in a Guinea pig model of heart failure. *Circ Res*. 2014;115:44–54. doi: 10.1161/CIRCRESAHA.115.303062.
9. Griffin TJ, Gygi SP, Ideker T, Rist B, Eng J, Hood L, Aebersold R. Complementary profiling of gene expression at the transcriptome and proteome levels in *Saccharomyces cerevisiae*. *Mol Cell Proteomics*. 2002;1:323–333. doi: 10.1074/mcp.M200001-MCP200.
10. Smith LM, Kelleher NL; Consortium for Top Down Proteomics. Proteoform: a single term describing protein complexity. *Nat Methods*. 2013;10:186–187. doi: 10.1038/nmeth.2369.
11. Chiang DY, Lebesgue N, Beavers DL, Alsina KM, Damen JM, Voigt N, Dobrev D, Wehrens XH, Scholten A. Alterations in the interactome of serine/threonine protein phosphatase type-1 in atrial fibrillation patients. *J Am Coll Cardiol*. 2015;65:163–173. doi: 10.1016/j.jacc.2014.10.042.
12. Krämer A, Green J, Pollard J Jr, Tugendreich S. Causal analysis approaches in Ingenuity Pathway Analysis. *Bioinformatics*. 2014;30:523–530. doi: 10.1093/bioinformatics/btt703.

KEY WORDS: heart failure ■ nitric oxide ■ proteomics ■ signal transduction ■ systems biology

Circulation Research

JOURNAL OF THE AMERICAN HEART ASSOCIATION



Seeing the Forest for the Trees Brian O'Rourke, Ting Liu and D. Brian Foster

Circ Res. 2016;119:1170-1172

doi: 10.1161/CIRCRESAHA.116.310066

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 2016 American Heart Association, Inc. All rights reserved.

Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:

<http://circres.ahajournals.org/content/119/11/1170>

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the [Permissions and Rights Question and Answer](#) document.

Reprints: Information about reprints can be found online at:
<http://www.lww.com/reprints>

Subscriptions: Information about subscribing to *Circulation Research* is online at:
<http://circres.ahajournals.org/subscriptions/>