Cardiac Systems Biology

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\textbf{ABSTRACT:} As more detailed molecular information accumulates on the biology of the heart and other complex systems in health and disease, the need for new integrative analyses and tools is growing. Systems biology and bioengineering seek to use high-throughput technologies and integrative computational analysis to construct networks of the interactions between molecular components in the system, to develop systems models of their functionally integrated biological properties, and to incorporate these systems models into structurally integrated multi-scale models for predicting clinical phenotypes. This review gives examples of recent applications using these approaches to elucidate the electromechanical function of the heart in aging and disease.

\textbf{KEYWORDS:} genomic phenotyping; heart; multi-scale modeling; systems bioengineering

\section{INTRODUCTION}

With the success of new technologies for genome sequencing, expression profiling, proteomics, structural biology, and \textit{in vivo} gene targeting, the reductionist emphasis of late twentieth century biology is now being complemented by a new emphasis on integrative biology in the twenty-first century and driven by advances in bioinformatics, systems science, and multi-scale bioengineering modeling.

It is helpful to begin with some working definitions: \textit{Bioinformatics} provides the tools to organize and synthesize information from extensive data on biological components into working knowledge and hypotheses. \textit{Systems biology} combines the molecular components of biological systems and subsystems into their interaction networks and formulates functionally integrated systems models for analyzing the input–output relationships of the networks and understanding how their emergent properties perform required biological functions. \textit{Multi-scale bioengineering} combines systems models with data on biological structure across physical scales and uses physico-chemical principles together with engineering methods to develop structurally and functionally integrative predictive models of the dynamic physiology of living systems in health and disease. The common theme is \textit{integration}. Therefore, bioinformatics facilitates \textit{data integration}; systems biology uses this
information to develop models that are functionally integrated across the molecular components of biological networks and across interacting physiological subsystems; multi-scale bioengineering develops models that are structurally integrated across scales of biological organization from molecule to organism or population.

The need for integrative analysis in cardiac physiology and pathophysiology is readily appreciated. Common heart diseases are multi-factorial, multi-genic, and linked to other systemic disorders such as diabetes, hypertension, or thyroid disease. Cardiac structure and function are heterogeneous, and most pathologies are associated with regionally inhomogeneous alterations in function. Even basic physiological functions such as cardiac pacemaker activity involve the coordinated interaction of many gene products in the cell, and indeed the interactions between heterogeneous cells and extracellular matrix. Cardiac pathologies with known molecular etiologies are often seen to depend critically on anatomic substrates for their expression in vivo; and of course there are many interacting subsystems at many scales involved in physiological processes. For example, substrate and oxygen delivery influence myocyte metabolism, which strongly affects myocardial mechanoenergetics, in turn altering ventricular stresses, which modulate coronary blood flow thus affecting substrate and oxygen delivery.

We summarize here some novel examples of data integration strategies for network discovery, functionally integrated systems models, and structurally integrated multi-scale modeling that have been used recently to investigate cardiac genotype-phenotype relations in acquired and genetic diseases and aging.

**HIGH-THROUGHPUT CARDIAC PHENOTYPING AND DATA INTEGRATION FOR GENE DISCOVERY AND NETWORK RECONSTRUCTION**

While we have lots of genomic and proteomic data, discovering their functions in a complex system like the heart is an exhaustive process often based on genetic manipulation and gene targeting in model organisms like the mouse. These approaches have been enormously informative, but a single transgenic mouse takes months to develop and study; a knockout mouse, over a year. The phenotypic consequences of genetic defects manifest gradually with age. Mice can live to three years of age, making studies very long and costly. Occasionally, two genetically engineered strains have been crossed to investigate pair-wise gene interactions. Back-crossing into different backgrounds has demonstrated the profound effects that genetic polymorphisms can have on phenotype, but does not immediately point to the interacting genes. Systematically profiling phenotypic variations in consomic mouse or rat strains—in which single chromosomes have been swapped between inbred strains—is one systematic approach to this problem, but this still requires the painstaking process of breeding and studying numerous congenic mice to isolate polymorphic alleles on the chromosome of interest.

In simpler systems, such as *Escherichia coli*, *Caenorhabditis elegans*, and *Saccharomyces cerevisiae*, that are amenable to large scale mutagenesis and rapid phenotyping, a strategy known as genomic phenotyping has been developed. When phenotypic characteristics can be assayed for genome scale mutant libraries, a large number of candidate alleles for further investigation can be found. Combining this
new phenotypic information with genomic or proteomic data can then be used to help discover novel networks and pathways. For example, in *S. cerevisiae*, Begley and co-workers\(^3\) used genome-wide phenotyping to identify hundreds of alleles important for cellular recovery after exposure to mutagens. By integrating the phenotypic data on cell recovery in over 1,600 gene deletion mutants with a database of the yeast interactome, they identified several multi-protein networks important for damage recovery. Some networks were associated with DNA metabolism and repair, but most were associated with unexpected functions such as cytoskeletal remodeling and lipid metabolism. In this way, systematic phenotypic assays can be linked to underlying molecular pathways.

Might this data integration approach be feasible for studying cardiac biology? There is reason to believe it might be. The fruit fly (*Drosophila melanogaster*) was the first organism with a functioning circulation to have its genome sequenced.\(^4\)

The heart of the fly consists of a tubular structure that contracts spontaneously throughout the insect’s lifespan and has the main function of circulating the hemolymph, which transports energy substrates from the abdomen to the thorax and head.\(^5\)

Several human disease models have been developed in *Drosophila*, particularly for neurological diseases.\(^6\)–\(^8\) *Drosophila* is also commonly employed as a model organism for studying the genetics of aging, partly because it represents a genetically tractable organism with a short life span.\(^9\) Genetic screens have allowed the identification of single genes that control life span in flies.\(^10,11\)

We made the first attempt to exploit *Drosophila melanogaster* for investigations of adult cardiac dysfunction.\(^12,13\) Lakatta commented that this work constitutes the “first big step” toward establishing *Drosophila* as a valuable new model in the study of cardiac aging.\(^12\) Studies of larval and pupal fly hearts, using different techniques, had been reported previously.\(^14\) We developed methods for studying cardiac function *in vivo* in adult flies. Using two different cardiovascular stress methods (elevated ambient temperature and external electrical pacing), we found that maximal heart rate is significantly and reproducibly reduced with aging in *Drosophila*, analogous to observations in elderly humans.\(^15\) The temperature stress is, in insects, a physiological stress on cardiac function. Insects are poikilotherms—their body temperature changes according to the environmental temperature. Therefore, a change in ambient temperature would affect the metabolic rate and require an increase in cardiac output. Enzymatic activity does indeed increase in insects with temperature over the range we studied.\(^16\) An increase in oxygen consumption has also been shown in *Drosophila* with increasing ambient temperature.\(^17\)

The main advantages of *Drosophila melanogaster* as a model organism are as follows:

- Large unbiased genetic screens can be performed.
- Genetic interactions can be identified by crossing the mutant of interest with collections of other mutants and identifying modifier genes (suppressors or enhancers).
- The short life span facilitates the study of the effects of aging.
- Many mutants and chromosomal markers are available.
- Genetic techniques are very powerful and have been perfected since the beginning of the last century.
• The genome has been sequenced, greatly speeding efforts to identify the genes responsible for mutant phenotypes.

The main disadvantage is the evolutionary distance from man. However, many important findings for human medicine and biology have originated from studies in Drosophila or other invertebrates. Examples are the identification of genes regulating embryonal development in Drosophila, the initial identification of several components of the apoptotic machinery, and elucidation of gene pathways involved in neurogenesis. In all these examples, rapid progress in the understanding of complex problems was made possible by initial investigations in Drosophila and subsequent extensions of the findings to mammals and humans. Several surveys have shown remarkable conservation of human genes in the fly genome, including cardiac disease-relevant genes.

The cardiac functional measurement obtained from the Drosophila model that is best suited for future studies in mice and humans is the decline of maximum heart rate with age. This is a very important index of cardiac function, as it is one of the main causes of the decrease in the capacity for physical work in older people. The other determinant of cardiac output, the stroke volume (the amount of blood pumped with each heart beat), does not change with age in humans. This age-related change is also present in rodents and it is easily measured in rodents and humans using non-invasive methods.

Scientifically rigorous human mortality trials for anti-aging interventions would require a very large population of subjects and to our knowledge have never been successfully performed. The problem is that the statistical power of survival analysis depends on the number of deaths within the study period. A physiological parameter such as maximal heart rate, however, could be measured in each individual, and therefore provide a more realistic end point for human studies.

Using a systems biology approach, after a complete list of the parts of the system is obtained, repeated cycles of modeling and systematic experiments take place. The three essential components of systems biology are

1. High-throughput technology to acquire biological data;
2. Biological tools for genome level perturbation studies, which are available in a few model organisms: yeast, Drosophila, C. elegans, and mouse; and
3. Quantitative models.

As an initial quantitative model, we recently proposed a computational model of aging that can explain several of the properties of aging genes, including their conservation across evolutionary distant species. The model is supported by an analysis of aging genes and interaction databases.

The broad spectrum of biological dysfunction that occurs during aging suggests that gene network level properties are especially important. We also showed that local network connectivity (the number of links per node) is significantly higher in aging genes as also reported by Promislow.

Many aging genes have been found from unbiased screens in model organism. Genetic interventions promoting longevity are usually quantitative, the number of genes involved is large, and the effect of the genetic background is strong. It is not clear how optimal interventions can be devised on such a complex genetics. In the case of aging research, therefore, the need for a quantitative model to guide and help interpret experiments is especially important.
The computational model presented in Ferrarini et al.\textsuperscript{26} suggests a general conclusion: restoring some nodes of a damaged network can have a large effect on the function of the network, uniquely because of their topological properties. In other words, higher connectivity might not just be a property of aging genes but actually the reason why they can affect the generalized dysfunction of aging.

This functional network model (based on network dynamics) can be further developed to allow the study of real biological networks and the integration of biological data. We are also developing high-throughput technology for the measurement of cardiac aging in \textit{Drosophila}. A systems biology approach can therefore be used for the study of cardiac aging in \textit{Drosophila}, iterating modeling and data collection.

**FUNCTIONALLY INTEGRATED SYSTEMS MODELS OF SIGNAL TRANSDUCTION IN CARDIAC MYOCYTES**

\textbf{Cardiac Myocyte Models}

The first cardiac myocyte models, published in 1960,\textsuperscript{28} used the formalism developed by Hodgkin and Huxley\textsuperscript{29} to model the contributions of sodium and potassium currents to the action potential (AP) and to investigate the mechanistic basis of the plateau of the cardiac AP. As new experimental data on myocyte electrophysiology were obtained, the models were refined and extended, and their results in turn informed new experiments. The latest generation of cardiac myocyte ionic models include upward of twenty ionic fluxes and forty or more ordinary differential equations. They also include separate compartments representing the sarcoplasmic reticulum (SR), the narrow subsarcolemmal dyadic space between the sarcolemmal dihydropyridine receptor and the ryanodine receptor on the SR, and the bulk myoplasm. But these compartments are lumped, and these “common pool” models have no spatial structure. They are functionally integrated systems models.

In addition to integrating across the cellular components responsible for ion fluxes and AP generation, systems models have also been developed that integrate the functional components of cellular subsystems responsible for calcium handling, myofilament interactions, and energy metabolism. This then creates the opportunity to develop systems models that not only integrate the components within a single subsystem but that also integrate the functions of two or more interacting subsystems. Excitation–contraction (E-C) coupling is one obvious such interaction, and mechanistic systems models of cardiac E-C coupling have been published since the early 1980s.\textsuperscript{30} Since then, more detailed models have been developed of myofilament activation by calcium and the effects of feedback from crossbridge binding.\textsuperscript{31} Bluhm et al.\textsuperscript{32} coupled the Luo-Rudy ionic model to a model of myofilament activation to predict the time-course of isometric tension development following an increase in sarcomere length.

This progress naturally suggests the integration of electrophysiological models with models of energy metabolism in the cardiac myocyte. The first comprehensive example was published by Ch’en and colleagues.\textsuperscript{33} The analysis gave valuable insights into the arrhythmogenic mechanisms of ischemia, especially during the highly vulnerable reperfusion period. More recently, a comprehensive thermokinetic model by Cortassa and co-workers\textsuperscript{34} analyzed control of cardiac mitochondrial bioenergetics.
by combining equations for the tricarboxylic acid (TCA) cycle, oxidative phosphorylation, and mitochondrial calcium handling. The model reproduced observations on mitochondrial bioenergetics, calcium dynamics, and respiratory control and demonstrated how calcium feedback provides a mechanism for matching mitochondrial energy production with the metabolic demand of the myocyte as workload changes. Michailova and McCulloch\textsuperscript{35} extended the model of the ventricular myocyte by Winslow \textit{et al.}\textsuperscript{36} by incorporating equations for calcium and magnesium buffering and transport by adenosine triphosphate (ATP) and adenosine diphosphate (ADP) and equations for MgATP regulation of the sodium-potassium pump, and the sarcolemmal and sarcoplasmic calcium pumps. Under normal conditions, the model showed that calcium binding by low-affinity ATP and diffusion of CaATP might affect the amplitude and time-course of intracellular calcium signals. Some of these predictions were subsequently supported by experimental observations.\textsuperscript{37} More recently, this model has also been used to study the effects of magnesium on ventricular excitation-contraction coupling.\textsuperscript{38}

### Systems Models of Signal Transduction Pathways

Computational models can help to provide a quantitative understanding of how signaling networks control cellular physiology. We distinguish between models of cell signaling at two levels of detail: large-scale top-down models and bottom-up mechanistic systems models. Each approach has advantages and disadvantages, while some hybrid approaches such as Bayesian network modeling offer advantages from each modeling strategy.\textsuperscript{39} Top-down modeling, often based on noisy proteomic or protein interaction data such as yeast two-hybrid studies, shows promise for understanding the qualitative properties of signaling networks.\textsuperscript{40,41} Top-down models may also be useful for discovering novel pathways.\textsuperscript{42} Mechanistic systems models typically begin by formulating biochemical reactions with kinetic rate laws as differential equations using available experimental data on describing reaction kinetics and parameters. They should be able to make quantitative, experimentally testable predictions.

\textit{In silico} analysis of network perturbations allows us to formulate new hypotheses regarding signaling pathways. Hoffmann \textit{et al.}\textsuperscript{43} used this approach to predict functional differences between IκB isoforms \textit{in silico}, and then used the model to design an experiment to test this prediction. Bhalla and Iyengar\textsuperscript{44} showed that feedback and crosstalk between neuronal signaling pathways could lead to emergent properties such as bistability, which may lead to long-term potentiation. When mechanisms are unknown and several hypotheses are plausible, systems models representing alternative hypotheses may be compared quantitatively. This approach was used to identify transitions between mode 1 and mode 2 gating of the L-type calcium channel during β-adrenergic stimulation.\textsuperscript{45}

However, mechanistic systems models are invariably data limited, especially for less well-characterized signaling networks. Optimizing a reduced model with sparse data is possible but often difficult. Inconsistency of experimental data from different sources may prevent any single model from predicting all data. While high-throughput genomic and proteomic approaches promise vast amounts of data, the data will be structural rather than functional. Mechanistic models of new signaling networks will require uniform high-throughput data and extensive validation against independent measurements.
While not yet as comprehensive as databases of metabolic networks, public data resources on cell signaling, such as the Alliance for Cellular Signaling, are emerging.\(^4^6\) (For another example, see http://www.signaling-gateway.org/.) This is opening the way for more detailed systems models of signal transduction pathways. By integrating models of signaling networks with models of cardiac myocytes, it is now possible to investigate neurohormonal regulation of E-C coupling. We developed a new model of \(\beta_1\)-adrenergic regulation of cardiac E-C coupling by combining a systems model of ionic currents and calcium handling in rat ventricular myocytes, with a novel mechanistic model of cAMP-mediated cell signaling via \(\beta_1\)-adrenergic receptor that included the L-type calcium channel, phospholamban, and inhibitor-1 as phosphorylation targets of protein kinase A (PKA).\(^4^7\) Later, we extended this model to include troponin-I and the ryanodine receptor (RyR) as additional PKA targets, though the analysis suggested that the controversial role of RyR phosphorylation during adrenergic stimulation may be fairly insignificant under normal conditions in the intact cell, owing to the effects of calcium autoregulation by the sarcoplasmic reticulum.\(^4^8\) This new class of models may be especially important for elucidating the pathogenesis of congestive heart failure, where dysregulated calcium handling in the myocyte is accompanied by downregulation of \(\beta\)-adrenergic signaling.

Cardiac cellular models are beginning to contribute to understanding phenotypic consequences of gene mutations linked to inherited arrhythmias,\(^4^9,^5^0\) notably long Q-T syndrome (LQTS), a family of mutations causing prolonged AP duration by affecting genes that encode the subunits of the fast and slow activating delayed rectifier potassium channels (\(I_{Kr}\) and \(I_{Ks}\)) and the fast sodium channel (\(I_{Na}\)). Of these, the most prevalent is LQT1, affecting \(I_{Ks}\), for which several specific defects in KCNQ1 have been identified in man. A complication in extending this work in reconstituted systems such as transgenic mice is that \(I_{Kr}\) and \(I_{Ks}\) are not present in mouse, a species which depends mainly on \(I_{to}\) and \(I_{Ksus}\) for repolarization.\(^5^1\) Therefore, computational analyses become more valuable and have helped elucidate arrhythmogenic mechanisms in LQT3,\(^5^2\) LQT2, and LQT6\(^5^0\) by incorporating biophysical properties of mutant channels (in heterologous systems) into ventricular cell models. Interestingly, in a study of LQT1 patients, 82% of lethal cardiac events occurred during exercise or emotional stress, suggesting a connection with sympathetic stimulation.\(^5^3\)

Recently, a mutation identified in a Finnish population (KCNQ1-G589D) was shown to disrupt binding of a PKA anchoring protein (AKAP9) known as Yotaio, to a leucine zipper motif near the C-terminus of the pore-forming subunit of the slow activating delayed rectifier potassium channel, \(I_{Ks}\).\(^5^4\) Yotaio was shown in this study to target PKA and phosphatase-1 to the channel. This mutation therefore selectively disables \(\beta\)-adrenergic regulation of \(I_{Ks}\), which is the main mechanism of APD shortening with increasing heart rate during sympathetic stimulation. To investigate how this defect may lead to ventricular arrhythmia during sympathetic stimulation, we reconstituted it in an \textit{in silico} model of beta-adrenergic signaling and E-C coupling in the rabbit ventricular myocyte.\(^5^5\) While the KCNQ1-G589D mutation alone did not prolong the resting AP when coupled with \(\beta\)-adrenergic stimulation in the whole cell, the mutation induced AP prolongation instead of shortening and after-depolarizations associated with reactivation of the L-type calcium channel. These changes are recognized cellular mechanisms for arrhythmogenesis.
Multicellular Models of Myocardial Electromechanics

Multicellular models come in three main varieties: cellular automata, resistively coupled networks, and continuum models. Cellular automata and resistively coupled networks use physical properties or rules that approximate them to combine individual cells modeled as systems of ordinary differential equations as described earlier. These models are computationally tractable and have been particularly informative in elucidating the effects of electrical loading by neighboring cells on the propagation of the AP from cell to cell via current flux through gap junctions. Continuum models of electrical impulse propagation typically use bi-domain or monodomain theory, in which the intracellular and extracellular conductivities of the tissue are lumped into diffusion tensors, which are anisotropic and may differ between intracellular and extracellular domains. Continuum methods are also widely used for modeling regional biomechanics in the heart and other tissues. Within the continuum framework, however, a constitutive model of the contributions of cellular and extracellular constituents is required to relate the passive and contractile stresses in the tissue to the state of deformation (strain). While many constitutive models are phenomenological relations, curve-fitted to multiaxial experimental measurements of tissue mechanical responses, it is possible to apply micromechanical principles and quantitative histological measurements to derive microstructural constitutive relations.

The development of anatomically detailed models of ventricular geometry and muscle fiber architecture in dog, rabbit, and pig, and atrial geometry and fiber bundle architecture in man has enabled investigators to develop structurally integrated continuum models of cardiac electrical impulse propagation and wall mechanics by using finite volume, finite difference, or finite element methods. As cellular models become more biophysically detailed and functionally integrated, the opportunity is arising for structurally integrated models that are also functionally integrated, such as continuum models of coupled ventricular electromechanics.

Returning to LQT1, it is apparent that the cellular defect alone is not sufficient to explain the incidence of malignant tachyarrhythmias. A growing body of evidence, especially from isolated perfused myocardial “wedge” preparations, suggests that transmural heterogeneity of cell type can lead to increased dispersion of repolarization and “phase 2 reentry” in the setting of drugs that prolong QT interval. In the absence of animal models, studies of drug-induced QT prolongation have also been the main model systems for understanding the mechanisms of malignant arrhythmias in the genetic LQTSs associated with loss of IKr and IKs function. Therefore, we incorporated the cellular model of the KCNQ1-G589D mutation into a three-dimensional continuum model of AP propagation in a rabbit ventricular wedge with transmurally varying muscle fiber orientations. While the KCNQ1-G589D mutation alone did not prolong the QT interval when coupled with β-adrenergic stimulation in the whole cell, the mutation induced QT prolongation and after-depolarizations, which are potential cellular mechanisms for arrhythmogenesis. These alterations amplified transmural repolarization heterogeneities in a three-dimensional rabbit ventricular wedge model and led to other T-wave abnormalities on simulated elec-
trocardiograms (Fig. 1). This model was is one of the first examples in which a single gene defect has been reconstituted into a ventricular tissue model computationally before it was done experimentally.

CONCLUSION

Bioinformatics, systems biology, and multi-scale bioengineering approaches are demonstrating potential to contribute to an integrative understanding of normal cardiac biology and the changes that occur during aging and genetic and acquired diseases. As computational power and high-throughput technologies continually improve, we will eventually be able to reconstruct and model more comprehensive and fully characterized cellular networks and use these systems models in multi-scale predictions of organ system phenotype in human heart diseases.
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