Published in final edited form as:

Drug Discov Today Dis Models. 2008 ; 5(3): 113–115. doi:10.1016/j.ddmod.2009.05.001.

Models that Buzz, Squeak, Ribbit and Oink: Using a Variety of Species to Study Cardiovascular Disease

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Study of the cardiovascular system by nature is a complex undertaking. Historically, the examination of the heart and vessels has taken an amazing course (no pun intended). For example Pliny the Elder (23–79 A.D.) wrote that that “The arteries have no sensation, for they are without blood...” while in 1628 William Harvey described the closed circulatory system of animals and man in his treatise, *Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus* (An Anatomical Exercise on the Motion of the Heart and Blood in Animals). Harvey himself studied a wide range of animals to draw his conclusions and noted “Oysters, mussels, sponges ... have no heart, for the whole body functions as a heart, and the animal itself is a heart.” He found his investigation so challenging that he said: ““When I first gave my mind to vivisections. ... I found the task so truly arduous, so full of difficulties, that I was almost tempted to think that the motion of the heart was only to be comprehended by God.” A few years later, however, in 1660, Malpighi used the frog to demonstrate blood movement in capillaries. In modern times, we have come to realize the importance of utilizing a wide range of organisms to study heart disease. For example, take the seminal observation by Rolf Bodmer (one of the authors in our series) that the homeobox gene *Msh-2*, renamed *Tinman* for the Wizard of Oz character, is required for the development of the primordial heart of the fly (1, 2). The murine ortholog of *Tinman*, *Nkx2-5* was subsequently cloned and mice lacking *Nkx2-5* were generated. Interestingly, the mutant mouse embryos display myogenic and morphogenetic defects in the heart (3,4). Mutations in human *Nkx2-5* were eventually identified in patients with a range of congenital cardiac defects, which frequently included atrioventricular conduction system abnormalities (5). Thus is clear that studying a range of species is advantageous for any scientific discipline, and is particularly important in cardiovascular biology.

With both history and cross-species science in mind, we can acknowledge and adapt Arnie Katz’s (6) historical flow which led to his “Paradigms in Study of Heart Failure.” These are:

1. Clinical observation (case study)
2. Anatomic observation (autopsy)
3. Circulatory and hemodynamic physiology

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4. Biochemistry, cell biology and biophysics
5. Molecular and developmental biology

The use of this paradigm might also be applied to our current series which we have assembled for the readers of *Drug Discovery Today: Disease Models*. Here, we hope to illustrate the importance of using a variety of approaches to study cardiovascular diseases, and how these necessitate a wide range of species that might in turn focus back on any or all of Katz' paradigms. Historically, these paradigms have been grounded in the physiological research on larger species, such as dog or pig. With the advent of advanced genetics and our ability to overexpress genes in the heart or vasculature, and more recently, reduce or delete gene function directly in cardiac myocytes, smooth muscle or endothelial cells, the mouse became the domineering model organism. The study of flies or frogs has always had its roots in biology, but as we have refined our genetic and molecular methods, and also miniaturized many of the technologies necessary to study the physiology of the cardiovascular system, they too play an expanding role in disease and drug investigations. Our mandate to each of our esteemed authors was to provide some illustration of the unique use of their particularly favorite species to probe the nuances of the cardiovascular system, and ideally make you, the reader, aware of the advantages of such a model system for drug discovery. As a brief introduction to our comprehensive species based approach, we provide the following.

In our first articles, we focus on the role of *Drosophila melanogaster*, the fruit fly, in cardiovascular research. Wolf and Rockman describe why this organism is an excellent model system to dissect the genetic pathways that are responsible for normal and abnormal cardiac function and why studying the adult fly heart can gain important information as to the basis of human heart failure. The authors review in detail the strategies to identify novel genes and pathways that cause or modify dilated cardiomyopathy. Then, Taghli-Lamalle and colleagues provide a companion viewpoint on using the fruit fly as an *in vivo* model system to study cardiac disease and, in particular, inherited cardiomyopathies. They give a detailed review on the rationale and the utility of *Drosophila* for identifying genes causally associated with cardiomyopathies, and how this unique model can probe their mechanisms of their action.

Chen and colleagues provide us with a comprehensive review of the use of zebrafish in the cardiovascular field. They describe the latest advancements in using this unique organism for the analysis of the cellular and genetic mechanisms regulating cardiac physiology and function. The authors link these latest developments to potential new avenues for drug discovery using the zebrafish.

Bartlett and Weeks discuss major features of the frog model and describe why *Xenopus* adds value by complementing studies in other organisms, particularly those with a focus on genetic and environmental influences on cardiac development and congenital heart disease. The authors describe the advantages of the *Xenopus* model and outline experimental manipulations that allow it to be used for testing the function of genes in heart development. Importantly, they make a case for using this organism for drug screening.

Evans provides a general overview of the current progress and challenges of cardiovascular stem cell biology. The author describes the merits and limitations of *in vitro* cardiovascular models and the current state of knowledge regarding genes and pathways regulating successive stages of cardiac specification, commitment, and differentiation gained from *in vitro* studies. Moreover, the author discusses background, progress, and potential clinical application of induced pluripotent stem (iPS) cells and the prospect of stem cell-mediated (or iPS-mediated) regenerative therapies for cardiovascular disease.

Atherosclerosis is the underlying etiology of coronary artery and cerebrovascular disease, the leading causes of mortality and morbidity in the developed world. In his review, deLuna describes traditional and novel mouse models used for atherosclerosis research as well as related research techniques. Based on these descriptions, it is easy to understand why the mouse is the predominant animal model currently used for atherosclerosis research.

Zaruba and Field discuss use of the mouse to study cardiac regeneration. They emphasize how the mouse system might be advantageous over other organisms to study the basic biology of this important, clinically relevant topic. The focus of the review is on novel strategies to induce myocyte proliferation, mobilize stem cells and how transplantation of cardiac myocytes might be made more efficiently, to permit these new technologies to be adapted to patients.

In his review, Dillmann provides a general overview of advantages and disadvantages to using rats as animal models for cardiovascular diseases. The author also reviews various rat models available for cardiovascular diseases and how to measure rat cardiac function *in vivo* and *ex vivo*.

Hypertension is one of the main risk factors for cardiovascular disease, stroke and chronic kidney disease. Ruiz-Opazo describes how rat models of hypertension recapitulate key events during the course of human essential hypertension. Moreover, the author provides compelling evidence why rat models of polygenic hypertension are ideally suited to provide a strategic platform for testing the safety and efficacy of anti-hypertensive therapies.

Pogwizd and Bers write a comprehensive review of the rabbit as a model of human heart disease. Their manuscript provides an excellent description of the advantages and disadvantages of the rabbit compared to other animal models. They also review different methods used in the rabbit model for both inducing disease states and for studying approaches to their treatment.

In the last review of this section, Coppola and Omens describe the unique advantages of large animal models over smaller ones, and why they still, to this day, remain invaluable for cardiovascular research. This manuscript reviews state of the art methods in assessing cardiac function at the whole organ level in large animals, such as measurements of mechanics, electrophysiology, and hemodynamics.

We hope that this comprehensive series, which outlines the advantages (and inferred, disadvantages) of a wide range of model systems for the study of cardiovascular diseases, is thought-provoking and educates the reader as to their utility for basic research and ultimately drug discovery. We apologize to those whose expert work could not be included here due to space limitations, and of course to those whose own favorite model system was not discussed.

With best wishes,

Robert S. Ross and Ju Chen

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Biographies

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Robert Ross is a graduate of Columbia College and the University of Southern California School of Medicine. Following internship and residency training in Internal Medicine at L.A. County-USC Medical Center and Cedars-Sinai, he was appointed as a Medical Staff Fellow in the Molecular Disease Branch of the NHLBI/NIH. Subsequently, he pursued additional post-doctoral training in molecular cardiology, as well as a clinical cardiology fellowship at University of California, San Diego, where he remained on faculty. Moving from UCSD to UCLA, he was appointed to faculty positions in Physiology and Medicine, and gained clinical

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Ju Chen received his B.S. in Genetics from Wuhan University, People's Republic of China. He received his Ph.D. in Molecular/Human Genetics from Indiana University Medical School in 1994. After his postdoctoral fellowship at UCSD in the Department of Medicine (1994–1998), he joined the faculty of UCSD and is currently a Professor of Medicine. His laboratory focuses on the understanding of the molecular basis of cardiac and skeletal muscle disease by utilizing genetically engineered mouse models, miniaturized physiological technology and a range of molecular and cell biological techniques.