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Hierarchical Approaches for Systems Modeling in Cardiac Development

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Abstract

Ordered cardiac morphogenesis and function is essential for all vertebrate life. The heart begins as a simple contractile tube, but quickly grows and morphs into a multi-chambered pumping organ, complete with valves, while maintaining regulation of blood flow and nutrient distribution. Though not identical, cardiac morphogenesis shares many molecular and morphological processes across vertebrate species. Quantitative data across multiple time and length scales have been gathered through decades of reductionist single variable analyses. These range from detailed molecular signaling pathways at the cellular levels to cardiac function at the tissue/organ levels. However, none of these components act in true isolation from others, and each, in turn, exhibits short- and long-range effects in both time and space. With the absence of a gene, entire signaling cascades and genetic profiles may be shifted, resulting in complex feedback mechanisms. Also taking into account local microenvironmental changes throughout development, it is apparent that a systems level approach is an essential resource to accelerate information generation concerning the functional relationships across multiple length scales (molecular data vs. physiological function) and structural development. In this review, we discuss relevant *in vivo* and *in vitro* experimental approaches, compare different computational frameworks for systems modeling, and the latest information about systems modeling of cardiac development. Lastly, we conclude with some important future directions for cardiac systems modeling.

Introduction

The first definitive organ to form in the embryo is the heart, whose morphogenesis, growth, and integrated function are essential to embryonic survival. The embryonic heart begins as a spontaneously contractile tube with an outer muscular (myocardial) layer and an inner endothelial (endocardial) layer. Myocardial and endocardial progenitor cells arise from bilateral populations of mesodermal cells close to the midbrain–hindbrain boundary [1]. Shortly before circulation is established, these mesodermal cells migrate medially to form a linear heart tube. This simple tube then undergoes complex morphogenetic movements that lead to the formation of a looped, multi-chambered organ complete with valves to regulate blood flow [2–4]. Though much research has highlighted roles of individual genes, the complex molecular networks that control this process are only beginning to be elucidated. Recent evidence suggests that the cardiogenic transcriptome is dependent upon the combinatorial binding of “master” transcription factors Gata4, Mef2a, Nkx2.5, and Srf. Together, these transcription factors have been targeted for regulation in over 1600 genes [5]. Only a small fraction of these genes have been investigated through the use of genetic

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mutant animal models, and filling this gap would consume significant time and resources. Each mutation causes compensatory changes in signaling networks that may further confound understanding, especially in less pronounced phenotypes. Genetic regulation is, however, only a fraction of the story. Cells are highly sensitive to microenvironmental factors (e.g. physiological conditions such as oxygen content or pressure load) which affect molecular targets across multiple scales (e.g. transcript levels, protein modifications, histone interactions) resulting in divergent effects [6, 7]. Deciphering the complexity is even more challenging when considering how features change in time (across development) and space (region of the heart).

Systems biology implements a process to identify emergent static and dynamic properties to obtain, replicate, and predict biological phenomena. The systems approach synthesizes experimental data into a computational model that not only simulates the previous data but can be used to predict previously untested interactions. These predictions in turn motivate further biological experiments; the results of which are used to then validate and improve the model [8, 9]. First, one must identify the core architecture driving a cellular process. A network is, technically speaking, a graph consisting of nodes connected by edges. In biological networks, nodes typically represent molecules like gene transcripts or proteins. Edges define the type of connection between nodes, e.g. interactions or relationships such as protein–protein interactions or transcription factor—DNA binding. Examples of biological networks include gene networks, transcription networks, protein–protein interaction networks, and metabolomic networks. This architecture can be derived from a variety of sources, such as primary literature and database websites. Relationships describing the node interactions include purely empirical, statistical, or defined by continuous equations (e.g. reaction-diffusion). Once the core architecture is in place, both high and low throughput, quantitative data from multiple single or small number factor, primary experiments are gathered. The model is then trained against the primary data to determine preliminary coefficient subsets to mirror the data, such that the biological networks provide physiologically relevant information. From the newly trained network, simulations can be conducted across possible parameter sets to prioritize and identify key regulatory nodes and recognize critical pathways through robustness analysis (Fig. 1). The predictions are then tested on follow-up biological experiments. These results can also be applied back to re-train the model to strengthen its specificity and predicative capacity.

Complex systems like the heart are composed of diverse elements constructed spatially and temporally in a hierarchical fashion [10]. Analyses of these multi-scale systems is likely most efficient by linking together different types of models that are ideal for particular scales. It is impossible and impractical to model the organ and tissue levels in the same framework as at the molecular and cellular levels, because they are driven by completely different stimuli that result in different responses[11]. A systems approach is necessary to connect, inform, and predict the functional relationships that span these length scales (e.g. molecular data versus physiological function and structural development) [12]. A cardiac developmental systems model has the potential to accelerate the discovery of key regulatory aspects of heart development and predict testable hypotheses about cardiac function eventually motivating new approaches for congenital malformations therapy. Although the necessary framework capable of integrating such massive amounts of data is still in its infancy, recent systems approaches have shown promise for enabling the identification of critical signaling protein complexes and, moreover, for predicting tissue morphogenesis through multi-scale approaches.

Experimental Models used to Generate Empirical Data

Generating suitable quantitative data is essential for developing a model with predictive capability. *In vivo* experimental and/or genetic perturbations of animal models, coupled with well-controlled in vitro analyses, have been vital to understanding mechanisms of cell and tissue level morphogenesis. Technology capable of generating large amounts of data across multiple experimental scales is relatively straightforward to apply; however, the technology that can be applied is dependent on the animal model. As a result, investigators often combine data from multiple animal models when performing computational studies [13]. A discussion of different animal models as well as appropriate corresponding experimental assays essential for connecting molecular and morphogenetic aspects of cardiac development proceeds below.

Zebrafish

Employed in research since the 1970's, the zebrafish (*Danio rerio*) is small, can be cultured inexpensively, and can produce up to 200 eggs per week as an adult. The zebrafish embryo is transparent, develops rapidly, and grows externally from the mother, which allows for ease in experimental manipulation and observation [14]. Zebrafish have a unique ability to develop in the absence of a functioning heart for at least 5 days post fertilization, since sufficient oxygen is obtained by diffusion [15]. Its genome has been fully sequenced, and zebrafish eggs have been found to tolerate microinjection and DNA delivery very well. Genetic manipulation via morpholinos and transgenics are one of the simplest of all vertebrate models [16, 17]. Furthermore, whole genome genetics and phenotype screens from mutagenesis agents can be filtered using a high throughput format [18, 19]. While the genetic process of myocardial formation of the zebrafish broadly parallels that of the human, the zebrafish only possesses a two-chambered, essentially right-sided heart. In addition, zebrafish valves do not develop through endocardial-mesenchymal transition (EMT) as in larger, higher order models [20]. Instead, the valves fold inward to create a thin, bilayered tissue without mesenchyme until much later [21]. Likewise, there are also differences in outflow tract/aortic arch morphogenesis compared to human morphogenesis [22]. The embryonic zebrafish heart is only a few hundred microns in length when fully grown, which limits non-genetic experimental inquiry and/or tissue isolation.

Amphibian

Amphibians, such as the African clawed frog (*Xenopus laevis*) and the eastern newt (*Notophthalmus viridescens*), have a three-chambered heart containing two atria, a single shared ventricle, and a shared outflow tract with a spiral shaped valve that helps partition relatively unmixed flow to the lungs and the rest of the body [23]. Amphibian eggs can be cultured very easily, but mesodermal cells are pigmented, limiting in vivo optical analysis during heart development stages. Morpholinos can be developed and employed in frog embryogenesis similar to zebrafish hearts [24]. Frog embryos were employed to show that cardiac laterality extends to the cellular level, with heterotaxic chambers and valves populated by cells from both sides [25]. Fewer studies have been conducted with the newt model, but recent findings show adult newt ventricular regeneration is similar to that of zebrafish suggesting it will become a more common model choice for heart development as its genome is clarified [26].

Avian

Avian models such as the chick (*Gallus gallus*) and quail (*Oreortyx pictus*) are well characterized and long applied in experimental embryogenesis ranging from cell isolation to *in-vivo* perturbation models. Within two to three days, chick embryos gastrulate, neurulate,

and fold into three-dimensional (3-D) animals with beating hearts, somites, and complex nervous systems. Complete cardiac morphogenesis in the avian model occurs in approximately 7 days, which is slower compared to other animal models and enables investigation of highly time sensitive transient features of morphogenesis such as septation and outflow tract morphogenesis [27]. Such rapid development is an advantage for experimental design and timely data collection. The anatomy of the chick heart closely parallels that of the human heart. In particular, the left ventricle, atrioventricular valves, and outflow tract valves are similar in shape, matrix composition, and cellularity. The principle differences between chick and human are that the right AV valve develops into a muscular flap valve in the right AV junction and the aorta arises from the right fourth aortic arch instead of the left [28]. Chick embryos can be cultured *in ovo* or removed from the eggshell [29] making them semi-transparent and of sufficient size to enable microscope guided experimentation [30, 31]. These experiments include microsurgery, tissue ablation, transplantation chimeras, local gene delivery, and biochemical delivery via soaked materials [32, 33]. Avian experiments have generated significant insight into the contributions of local cell populations to tissue morphogenesis and physical function. Although genetic manipulation is more technically challenging and time consuming in the avian embryo, some transgenics are available for study [34].

Drosophila

The fly (*Drosophila melanogaster*) is one of the most intensively studied organisms in biology and has served as a model system for the investigation of cardiac development. The *Drosophila* heart (also called the dorsal vessel) is an organ for hemolymph circulation that resembles the vertebrate heart at its transient linear tube stage. Dorsal vessel morphogenesis shares several similarities with early events of vertebrate heart development. In particular, cardiac progenitors are derived from laterally positioned mesoderm, being specified through the use of conserved cellular induction pathways and downstream transcriptional effectors [35]. At these early stages of heart development, the drosophila has been a widely used system for the study of cardiogenesis due to its relatively simple structure and the productive use of *Drosophila* genetic approaches [36]. Genetic analyses have identified hundreds of important intracellular transducers, which provide large gene regulatory networks essential for mapping the underlying cardiac specification [37]. Currently, projects such as the Berkeley Drosophila Genome are in the middle of a sustained effort to define the expression patterns of all predicted genes in the genome [38]. This information will be critical in identifying what genes contribute to the cardiac phenotype.

Rat

The rat (*Rattus norvegicus*) has been used extensively in cardiac research, although studies focusing on embryonic development have been limited. This is largely due to the abundance of genetic models available in mice and the overall economic cost. The majority of investigations focus on later stage pathogenesis of cardiovascular disease and/or the effects of drug intervention [39]. In general, the rat embryo, fetus, and postnatal morphologies correlate with both mouse and human development, and nearly all the features (external and internal) of the embryonic chick [40]. This provides an excellent morphological and chronological model for all stages of cardiac development. A major advantage of the rat is the relative ease with which surgical techniques can be performed when necessary for some disease or treatment models [41]. In many cases, measurement of relevant circulatory, biochemical, and hemodynamics parameters have been largely optimized for understanding cardiac function.

Mouse

Mice (*Mus musculus*) are the mammalian animal model of choice for cardiac developmental genetics. The mouse has a relatively fast gestation cycle, many pups per litter, and relatively short post-natal development compared to other mammals. Nearly every genetic tool developed has reagents available for the mouse. Mice tolerate oocyte injections very well, and their embryonic stem cells receive DNA easily. Unlike the other animal models, mice have a placental circulation and a fully four chambered heart with correct laterality, similar to humans [42]. However, the chordae of the murine AV valves are far less prominent than the pronounced tendinous chords of the human heart valves and heart beat frequency during development is 6-8 fold higher [28]. The cellularity and matrix striation of mouse valves are significantly less prominent than in humans or avians, in part because of their small size. Mouse embryos are difficult to culture outside of the mother, precluding experimentation except during the earliest stages of cardiogenesis [43]. Quantifying mouse heart physiology has also been challenging because of its size and speed with investigators normally relying on approximations from the dorsal aorta.

The largest integrated database of genetic, genomic, and phenotypic data has been generated for the laboratory mouse and compiled by the Mouse Genome Database (MGD) [44]. Reporter genes, such as *lacZ* or GFP, provide a sensitive method of detecting and following gene expression trends during morphogenesis. Mutant mouse transgenesis, either to overexpress or knock out specific genes, is an indispensable component of current research [45, 46]. Tissue-specific promoter targeting constructs, especially when combined with conditional expression systems such as Cre/Lox, has permitted spatial and temporal mapping of gene mapping and perturbation through manipulation [47-49].

Overall, each animal system has advantages and limitations to studying cardiogenesis, and no one model yet captures all components. A key challenge with systems approaches is merging and analyzing data acquired from multiple animal models, especially in situations where the morphogenetic processes are known to be different (Table 1).

In Vitro Experimentation

In vitro culture systems enable well-controlled investigation of fundamental molecular, cellular, and tissue level responses in isolation from other tissues. These experiments require the ability to isolate pure tissue segments that can either be explanted directly or digested to obtain cells. Genetic manipulation of cells and tissues, including overexpression and knockdown, is achieved through a variety of methods including electroporation, viral delivery, or lipid carriers [50-52]. The vast majority of genetic manipulation studies have used isolated cells cultured on artificial plastic substrates. Several studies have recently demonstrated that the physical properties of the cellular microenvironment (*e.g.* stiffness) can have profound effects on cell phenotype [53]. Tunable soft culture substrates have been developed to overcome these limitations, most notably polyacrylamide and polydimethylsulfone (PDMS) gels [54]. These materials can be further functionalized via microfabrication and selective polymerization techniques to exhibit gradients in stiffness, adhesion ligands, growth factors, and even gene plasmid concentration [55]. While microfabrication and selective polymerization approaches enable high throughput screening of the effects of the different gradient parameters on cell phenotype, these model systems remain inherently two dimensional (2D). Three dimensional (3D) culture models can be created using tissue engineering techniques, mainly with aqueous hydrogels created from natural extracellular components, which include collagen, fibrin, alginate, and hyaluronic acid [56]. The addition of hybridizing biopolymers and synthetic polymers can enhance the range of possible material properties [57]. One of the first examples of a tissue engineering

approach used a collagen hydrogel to study embryonic heart valve formation. Runyan and Markwald *et al.* isolated avian AV canals (chick HH14- or mouse embryos E8.5) and then explanted onto the surface of a collagen gel surface [58]. It has since become a staple assay to study 3D matrix interactions in developmental biology.

Genomics and epigenomics

The emerging epigenomic changes and the interactions between *cis*-acting elements and protein factors may play a central role in gene regulation [59]. To understand the crosstalk between DNA and protein on a genome-wide scale, techniques like ChIP-seq take the approach of combining chromatin immunoprecipitation with microarray technology [60, 61]. This high throughput strategy helps screen the targets of critical transcription factors and profile the genome-wide distribution of histone modifications, which will enable the feasibility of conducting large-scale studies, such as the Human Epigenome Project [62]. Many variations of the CHIP technology have been implemented such as the presence of DNA methylation (MeDIPchip) or DNA methylation based on the hypersensitivity to DNaseI cleavage (DNase-chip) [63, 64]. Other applications include the analysis of sequence variations by genotyping microarrays, copy number variations (Array-CGH), or RNA profiling (mRNA, microRNA). A custom-made, cardiovascular-based cDNA microarray, termed the CardioChip, was created to characterize gene expression in end-stage cardiomyopathies relative to gene expression associated with heart function [65]. Even more recent, NGS technology (NGS) has revolutionized the genomic and transcriptomic approaches to biology reducing the sequencing cost and significantly increasing the throughput [66]. It is expected that NGS will become more important in the study of inherited and complex cardiovascular diseases (CVDs) [67]. However, the NGS approach to the genetics of CVDs represents a territory, which has not been widely investigated.

These approaches have been useful for characterizing expression profiles in developmental, normal, and diseased conditions [68]. For example Chen *et al.* and colleagues found that *Csx/Nkx2-5*-deficient hearts were hypertrabeculated and noncompacted at birth. At adulthood, the mice exhibited progressive heart failure characterized by chamber dilatation with extensive trabecular formation [69, 70]. Upon microarray analysis, aberrant expression was revealed in a number of genes including the potential downstream targets, such as bone morphogenic protein-10 (BMP-10). A similar approach was used to understand the existence of regulatory pathways active in developing AV valves and bone progenitor cells. Overall, MC3T3 cells (pre-osteoblasts) were significantly more similar to late stage valves (E17.5), supporting the hypothesis that valve maturation involves the expression of many genes also expressed in osteoblasts [71].

Computational resources for data integration and model implementation

The progressive accumulation of enormous amounts of heterogeneous molecular response data has created a significant need to create databases that can organize, store, and rapidly retrieve pertinent information. In this section we will discuss the organizational framework necessary to approach a systems level question: (1) integration of heterogeneous datasets from a wide spectrum of databases, (2) standardization of systems languages to facilitate data representation and interoperability, and (3) robust integrative software tools available for systems biology research.

Biological Databases

The emergence of large-scale biological information has driven the development of global databases. These databases are important for the future of systems biology, as they centralize quantitative and qualitative data, ranging from protein structures to signaling pathways.

There are organism specific databases available containing detailed gene expression data of heart development for zebrafish [72], chicken [73], and mouse [74] : ZFIN, GEISHA, and GXD, respectively. However, to fully take advantage of these databases (biological information in general), one would need to obtain repeatedly the information necessary to piece together the larger network, which is not at all practical [75, 76]. A comprehensive database which integrates not only “-omics” datasets from experiments and literature, but also other information such as kinetic data and existing mathematical models is invaluable given the resources currently available to researchers [69, 77-79]. There are many resources for integrated data analysis as reviewed by Joyce and Palsson [80, 81]. To name a few, HCNNet [82], GEO database [29], and Cardiac Integrated Database Management System (CIDMS) all contain information related to genetic regulation of heart development and function. ArrayExpress is also becoming a popular database, largely due to the minimum information about micro-array experiment (MIAME) format which provides succinct and reproducible high-throughput functional genomics data [83].

Multi-scale standardization of system biology languages

As previously mentioned, a major problem in systems biology is establishing and maintaining cohesion between data derived from multiple independent experiments that may use different cell sources with slightly different controls, doses, time-courses, and analysis methods. Standards for the interpretation, handling, and dissemination of data should be given priority. It is crucial that individual research groups be able to exchange their models and create publicly accessible data banks and software environments that function with a broad selection of tool sets. There are many data exchange formatting standards currently available to aid in the exchange of information such as the MIAME [84], Proteomics Standard Initiative- Molecular Interactions (PSI-MI) [85], Biological Pathway Exchange (BIOPAX) [86], and Minimum Information Required In the Annotation of biochemical Models (MIRIAM) [87]. Systems Biology Markup Language (SBML) [88], Cell Markup Language (CellML) [89], and Field Markup Language (FieldML) [90] are machine-readable formats and standard forms of information exchange between software tools that provide both spatially distinct qualitative and quantitative models of biochemical networks (Sauro and Bergmann 2008; Kohl 2011). On the other hand, ontologies are well structured, controlled platforms providing standard definitions and concepts, as they aid in facilitating semantic-level data integration. Systems Biology Ontology (SBO) and Systems Biology Graphical Notation (SBGN) projects are also gaining popularity. For example, ontologies are important to define spatial domains at both the molecular and cellular levels (GO-MF- Gene Ontology Molecular Function, CBO-Cell behavior Ontology) while their function may be determined by the physics of biology and/or phenotype and traits (OPB-Ontology of Physics for biology, PATO-Phenotype and Traits Ontology). However, the Extensible Markup Languages (XML) described above have been adopted as standards for representing biological data despite the limitation in data interoperability. There are multiple resources available that discuss the topics mentioned above in depth [91-93].

Example approach for multi-scale simulation of valvulogenesis

Many examples illustrating a systematic approach could be taken from heart development, but for this purpose we will discuss heart valve morphogenesis, as it is a fundamentally important component of cardiac development and a process for which significant data exists characterizing the anatomical morphogenesis, microstructural remodeling, and molecular regulation [94] (Fig. 2). Briefly, valvulogenesis initiates through a process called endocardial-to-mesenchymal transition (EMT) by which the endocardial lining in specific regions of the linear heart tube (in the AV canal and outflow tract segments) differentiate into a mesenchymal phenotype and invade into the gelatinous matrix underneath called the

cardiac jelly. EMT is characterized by loss of tight junctions, downregulation of cell-cell adhesive contacts (*e.g.* E-cadherin, PECAM1), upregulation of cell-matrix adhesions (integrins), and development of contractile cytoskeletal machinery [95]. Continued cellularization and expansion of these tissue regions creates apposing pairs of tissues dubbed “cushions” that act as primitive valves to maintain unidirectional blood flow [96] [97]. This cellular transformation of EMT has also been shown in other processes such as fibrosis and oncogenesis [98].

The need for systems biology is becoming more apparent as the complexity of EMT—both spatial and temporal fashion—remains to be fully elucidated [99]. By applying a standardized strategy through XML based languages and systematic ontologies, centralization of ideas, resources, and strategies will be critical for continual advancement in the appropriate prediction of complex tissue formation such as valvulogenesis. In Fig. 2 the different levels of spatial and temporal scales as they pertain to heart development and their associated modes of representation are illustrated. A signaling network within the cell may be represented using an ordinary differential equation (ODE) within a markup language, such as SBML, that enables the network to be interpreted by many different modeling platforms. At the next level up, cell-cell interactions and their chemical signaling mechanisms can be represented by partial differential equations (PDEs) or Stochastic Petri Nets. For larger cell networks at the growing tissue level, some form of agent based modeling (ABM) is likely to be used. When looking at the level of the developing heart and its structural components, a finite element (FE) approach or a multiphysics simulation can be used to understand the functional relationship between the different components of the heart from its walls, valves, and vessels.

Integrative software

There are multiple approaches available in the research community used to facilitate integration of cardiac systems data. The most useful programs (1) handle genome-size data sets, (2) integrate multiple data types obtained from systems measurements, (3) provide a way to map and model networks and pathways, and (4) contain a user friendly interface as well as visual display of both the data and models. We focus on software tools that do not require the individual to have an extensive background in computer programming but still prove to be valuable resources for cell biologists. A few software tools that have been proven useful in the integration and visualization of biological networks are Cytoscape [100, 101], NAViGaTOR [102] VisANT [26] and Pathway Assist [103]. Other software specifically used for cardiac modeling include CellDesigner which is a structured diagram editor for drawing gene regulatory and biochemical networks stored in SBML for use in simulation packages [88, 104]. Through a graphical user interface, the dynamics of the network under defined input parameters can be observed [105]. Likewise, Continuity 6 is a problem-solving environment for multi-scale modeling in bioengineering and physiology, especially cardiac biomechanics, transport and electrophysiology, and it is distributed free for academic research [106].

Even though multiple data sources are available, most software programs can only access one or two of these databases, creating limitations. For instance, functional data sources (*e.g.* GO and literature) are incompletely annotated and biased toward better-studied genes; whereas, sequence databases have been used only to produce general disease probabilities. Integrative data analysis strategies have been proposed for the prioritization of genes involved in a given biological process, phenotype, or disease [107, 108]. One such software tool developed by Aerts *et al.* is a gene prioritization method; while providing the user control over the selection of training genes, it prioritizes both known and unknown genes,

ranks genes involved in human diseases and biological processes, and it uses rigorous statistical methods to fuse individual rankings into an overall rank and probability [109].

To bridge the gap between analysis strategies and clinical genetics, Barriot *et al.* integrated a candidate gene prioritization method and browsing of networks of gene interactions into a Wiki platform for the genetic study of CHDs termed CHDWiki [110-113]. The CHDWiki portal focuses on mapping out the gene network leading to human CHD phenotypes. It supports both genetic and molecular biology research that aims at searching for CHD genes, as well as clinical research that aims at identifying and interpreting genetic aberrations in patients who suffer from well-characterized CHDs. Comparative genomic hybridization (CGH) was the first efficient approach to scanning the entire genome for variations in DNA copy number. In a typical CGH measurement, total genomic DNA is isolated from test and reference cell populations, labeled differentially, and hybridized to metaphase chromosomes or, more recently, DNA microarrays [114]. A useful sequencing technology, exome-sequencing, has obvious utility as an important diagnostic tool for disorders that are characterized by significant genetic heterogeneity. However, its diagnostic utility is also becoming evident in the face of wide, and previously unsuspected, phenotypic heterogeneity such as CHD [115].

Computational Modeling of Cardiac Development

A particularly challenging aspect of understanding the development process of cardiac formation is the complexity of information at multiple spatial scales evolving in a dynamic manner; thus, a systems approach to understand the flow of information is critical. As we move forward with cardiac model generation pertaining to cardiac development, there is considerable debate over the best strategy for biological simulation, whether it should be “bottom-up,” “top-down” or some combination of the two [116, 117]. The consensus is that the approach be “middle-out,” meaning that we start modeling at the level(s) at which there are rich biological data and then reach up and down to other levels [118]. In the case of the heart, we have benefited from the fact that multiple levels of quantitative data have been gathered ranging from detailed molecular signaling pathways at the cellular levels to complex 3D geometrical models of the whole organ [119, 120]. Connecting these structurally diverse and hierarchically spatial levels of interactions has been challenging. We will now discuss current cardiac models involving molecular regulation of the cardiac transcriptome, morphogenesis, and function.

Molecular Mechanisms Regulating the Cardiac Transcriptome

With the emergence of the human genome project and advancement of biotechniques, detailed signaling networks and systems models describing these networks have started to generate momentum. Recently, a group sought to map the molecular patterns critical to cardiogenesis through interrogated gene expression in stem cells undergoing guided differentiation, while generating genomic paradigms responsible for confinement of pluripotency. They found that examination of gene relationships revealed a non-stochastic network anchored by integrin, WNT/ β -catenin transforming growth factor β and vascular endothelial growth factor pathways, which were validated by manipulation of selected cascades that promoted or restrained cardiogenic yield. Moreover, candidate genes within anchor pathways acted as nodes that organized correlated expression profiles into functional clusters which collectively orchestrated and secured an overall cardiogenic theme [121].

Underlying these signaling pathways, a combination of key transcription factors was found to drive a wide range of gene expression essential for cardiac development [69, 122]. Using a custom Chip-chip analysis (promoter and enhancer sequence) and cardiac myocyte mRNA expression levels, it was found that the combinatorial contribution of Gata4, Mef2a, Nkx2.5,

and Srf transcription factors was responsible for regulating the cardiac transcriptome. Specifically, the 91 target genes were bound by all four factors, 121 target genes were bound by three, and 286 target genes were bound by two transcription factors, all targeting over 1600 genes. Surprisingly, Gata4 and Nkx2.5 had the lowest number of targets (345 for Gata4, 276 for Nkx2.5), but it was found that their co-binding to 143 genes made each transcription factor highly correlated [5] (Fig. 3).

Furthermore, histone modifications and microRNAs modulate the underlying functional consequence of these cardiac transcription factors. Histone-3 acetylation was found to have a strong correlation with Srf- and Gata4- dependent gene activation. Moreover, upon Srf knockdown, a large proportion of targets were found to regulate microRNAs which may potentially explain up to 45% of indirect mRNA targets, thus representing an important intermediate layer of regulation [5]. Interestingly, it was found that even these non-paralogous transcription factors can partially compensate each other's function, as shown through RNAi knockdown experiments. In addition, enzymatic complexes such as the Swi/Snf-like BAF complexes remodel chromatin to allow the transcriptional machinery access to heart-specific enhancers [123]. Through experimental observations, it was revealed that dynamic randomness of SWI/SNF occurs in the *cis* chromatin remodeling process. By applying a stochastic model, a recent group identified the physiological properties of the chromatin remodeling process through a system of parametric microevents. Their simulations showed that SWI/SNF chromatin remodeling has low energy efficiency suggesting that the orchestrated chromatin remodeling makes few kilobase-pairs of the DNA accessible to the transcription machinery in a timely manner [124].

Cardiac Signaling Networks and Morphological Changes

Connecting these underlying molecular networks that drive cardiac tissue morphogenesis is important for understanding aberrations, which underlie a wide spectrum of human disorders including congenital cardiac defects. A recent study sought to connect model human heart morphogenesis through dissecting the underlying functional signaling pathway. Using detailed phenotype information from deleterious mutations in 255 genes with high-confidence experimental interactome data, systematic analysis of spatio-temporal protein networks driving cardiac development was revealed. A striking temporal correlation between organ complexity and the number of discrete functional modules coordinating morphogenesis was found (Fig. 4). For example, abnormal myocardial trabeculae formation was found dependent upon core networks within the NOTCH, Focal Adhesion, and ERBB signaling pathways, while abnormal valve formation was found dependent upon core pathways within the ERBB, Retinoic Acid, and Neurotrophin signaling pathways [125].

A follow up study explored the relationships between congenital heart disease (CHD) risk factors and responses using datasets from similar studies of CHD in humans and model organisms. They found that CHD risk factors functionally converge in protein networks driving the development of specific anatomical structures (*e.g.* outflow tract, ventricular septum, and atrial septum) which are malformed by CHD. This integrative analysis of CHD risk factors and responses suggests a complex pattern of functional interactions between genomic variation and environmental exposures that modulate critical biological systems during heart development [126].

Interestingly, recapitulation of these critical signaling pathways were shown when murine embryos were exposed to high glucose, a condition known to induce cardiovascular defects in both animal models and humans. Through a mass spectrometry-based proteomics approach, unique protein clusters were found differentially expressed in embryos with congenital defects when compared to normal developing embryos. These protein clusters

were mapped to adhesion/migration, differentiation, and insulin signaling pathways while correlating with increased protein levels observed in the amniotic fluid of human fetuses with CHD [127].

Toenjes *et al.* and colleagues used a similar approach to precisely describe the variety of heart malformations observed in the patients using an integrative approach combining cardiac gene regulatory networks based on correlated gene expression and optimized prediction of transcription factor binding sites. From this combined approach, they were able to find genes that appeared to be specifically associated with certain phenotypes such as Tetralogy of Fallot (TOF), transposition of great arteries (TGA), or ventricular septal defect (VSD) [128].

Modeling Cardiac Function

Numerous models for cardiac function have been based on proper physio-chemical and/or biophysical principles that emphasize different levels of structural organization. Contrary to signaling networks, cellular networks use physical properties or rules that approximate individual cells into large scale combinations to form systems [77]. These models are computationally tractable and informative since predictability using intrinsic physical properties has proven reliable. For example, dynamic gap junction models coupled with cardiac cell models have proven insightful in discerning how Ca^{2+} transients affect gap junction conductance during action potential propagation leading to potential arrhythmias [50, 51, 129]. Likewise, dynamics in heart trabeculae with changing heart frequency (*e.g.* mechanical load increases during development) was found to support the hypothesis that mitochondrial matrix Ca^{2+} plays an important role in matching energy supply with mechanical demand in cardiac myocytes [130]. Other systems have focused solely on the mechanical environment leading to proper heart tube function and formation. For example, heart tube formation is driven by cytoskeletal contraction, as exposure to the myosin-II inhibitor blebbistatin arrested any shortening and also decreased both tissue stiffness, measured by microindentation, and mechanical tension, measured by cutting experiments. Finite element (FE) simulations complementing these experiments suggest that the endoderm—not the mesoderm—is the primary contractile tissue layer during this process [131]. Specific to embryonic heart valves, Buskohl et al. developed a computational model of avian embryonic AV valve (cushion) growth and remodeling using experimentally derived parameters for the blood flow and the cushion stiffness. Interestingly, they found that the pressure distribution on the AV cushion was sufficient to generate leaflet-like elongation in the direction of flow, through inducing tissue resorption on the inflow side of cushion and expansion on the outflow side. Meanwhile, shear tractions minimally altered tissue volume, but regulated the remodeling of tissue near the cushion surface, particular at the leading edge [132].

Modeling the whole heart typically requires a continuum approach such that these models attempt to explain the integrated function in terms of ventricular anatomy, the structure and material properties of myocardial tissue, and/or associated intra-cellular and extracellular conductivities of the tissue [133]. With the development of anatomically detailed models of ventricular geometry and associated muscle fiber architecture, models have been developed to incorporate cardiac electrical impulse propagation [134]. For example, physiologically based mathematical models of the ventricular cell, together with a realistic 3D computer model of cardiac anatomy, predicted that dynamical instabilities remain a major cause of the wavebreak that drives fibrillation, even in an anatomically realistic heart [76]. Others have incorporated further model complexity by integrating continuum models of cardiac electrical impulse propagation and wall mechanics through finite element methods (FEM) [135, 136].

These models have proven useful in understanding relationships between ventricular contraction and heart dilation, an important component of cardiac development [34].

Limitations and Future Directions

Cardiac development is inevitably multi-scalar and contains elements of a diverse nature constructed spatially in a hierarchical fashion. To understand this process and the origins of congenital heart defects, an integrative approach is needed to connect a diverse range of biological information and computational frameworks within a spatial hierarchy. However, some of the fundamental limitations with multi-scale analysis are biological uncertainties, lack of integrating the diverse modeling environments, and unimaginably large computational times [137, 138]. To combat these limitations, most of the systems insight has been implemented through focused and directed models such as cardiac transcriptome networks or protein-clusters correlating with tissue morphogenesis. These have been extremely useful for understanding cardiac differentiation and connecting aberrant tissue morphogenesis to congenital heart defect.

Modularity of conserved canonical biological frameworks may help bridge these gaps [139]. Having such units well defined provides for security in archiving, in model sharing, and in ease of reproducibility and selection in model construction. It also renders those units more accessible and independently modifiable. We see such modularity approaches to combine functionally integrated cell systems models of cardiac metabolism, signal transduction, and excitation-contraction, coupling interactions into multi-scale models of cardiac electromechanical interactions that include hierarchical structures (*e. g.* cells, tissue, organs) [140, 141].

Recently, a number of groups have already sought to undertake the challenging tasks of incorporating many of the above mentioned processes into multi-scale models [119, 136, 141]. Through the generation of powerful software tools, integration of computational models for interpreting functional or clinical significance is starting to be achieved. Continuing on with this process, the need for open source web technologies and associated informatics approaches (*e.g.* Continuity 6) are important for improvements in data sharing, integration, and mining. This includes data representation (markup language, graphical), computational algorithms, and incorporating mechanical aspects such as FEA analyses packages.

In whole, connecting the phenotype of cardiac formation with the underlying molecular regulation is a grand challenge. Using both “top-down” and “bottom-up” approaches, insightful information regarding tissue morphogenesis and underlying transcription networks is being achieved [142]. As improvements within the field are made, investigations can begin to integrate the spatial hierarchy of cardiac development and to tackle difficult biological problems on unprecedented scales. In time, we may fully understand the origins of genetic and environmental factors leading to the majority of CHD and devise strategies for novel therapeutic and more importantly, preventive approaches.

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Figure 1.

The objective of systems biology is to integrate low- and high-throughput data with predictive computer modeling to better understand the properties of networks and cell systems important to human health. To apply such an approach, one must identify the core architecture driving a cellular process through biological networks, gather both high- and low-throughput quantitative data from single factor experiments, train and validate the model for physiological relevance, and run simulations in order to prioritize wet experiments.

Figure 2.

Heart valve morphogenesis initiates through a process called epithelial-mesenchymal transition (EMT), in which endocardial cells lose cell-cell contacts (*i.e.* tight junctions), acquire cell-matrix adhesions, and undergo cytoskeleton rearrangement. The modeling framework encompasses a wide range of spatial scales 10^{-9}m (*e.g.* proteins) to 10^{-3}m (*e.g.* the primitive heart tube), and temporal scales from 10^{-6}s (*e.g.* molecular events) to 10^6s (*e.g.* weeks of heart development). Multiple XML languages (*e.g.* SBML, CellML, FieldML) and ontology's are required for multi-scale modeling of this complex tissue formation during development. Figure adapted from [143].

(PRO-Protein Ontology, ChEBI-Chemical Entities of Biological Interest, CL-Cell Type Ontology, FMA-Foundational Model of Anatomy, GO-CC-Gene Ontology Cellular Component, GO-MF-Gene Ontology Molecular Function, CBO-Cell Behavior Ontology, OPB-Ontology of Physics for Biology, PATO-Phenotype and Trait Ontology, GO-BP-Gene Ontology Biological Process)

Figure 3.

Transcription factor network showing a selection of cardiac relevant genes (nkx 2.5, srf, gata4, mef2a) bound in ChIP-chip and/or ChIP-seq, and differentially expressed in RNAi knockdown experiments of the respective factor. Up- and down-regulation of genes is depicted and occurrence of ChIP binding marked by color-coded circles. Figure taken from [5].

Figure 4.

Examples of four functional networks driving the development of different anatomical structures in the human heart. These four networks were constructed by analyzing the interaction patterns of four different sets of cardiac development (CD) proteins corresponding to the morphological groups ‘atrial septal defects,’ ‘abnormal atrioventricular valve morphology,’ ‘abnormal myocardial trabeculae morphology,’ and ‘abnormal outflow tract development’. Centrally in the figure is a haematoxylin-eosin stained frontal section of the heart from a 37-day human embryo, where tissues affected by the four networks are marked. Figure taken from [125].

Table 1
Summary of advantages and disadvantages of animal models in the study of cardiac development.

Model organism	Defined cardiac morphogenesis staging	Anatomical similarity to human heart	Ease of genetic manipulation	Ease of experimental manipulation	Ease of in vivo observation
Zebrafish	+	+	++	++	+++
Amphibian	+	++	+	+++	++
Avian	+++	+++	+	+++	+++
Mouse	++	+++	+++	-	+
Drosophila	-	-	+++	-	-
Rat	+++	+++	+	+	+