Multiscale physiological systems

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Descriptions of systems and their power for explanation and interpretation are limited when the framework in which they operate is restricted to one level of scale. In fact, the majority of work in all disciplines is afflicted by this restriction. Using methods, tools and computing power now available, multiscale systems descriptions have become possible and are beginning to appear in the literature. This paper adds to that body of work by investigating the developing embryonic heart from three levels of scale: anatomic, cellular and protein. Pointers to subprotein levels of scale are indicated, which are not restricted to a genetic level description. A link to practical use of the findings is established by considering a specific condition that affects the newborn—the tetralogy of Fallot.

1. Introduction

There is a powerful systems level argument for the further development of multiscale phenomena and the continued understanding they provide in a systemic analysis of physiological systems. In science and engineering it has become the norm to tame complexity by the use of reductionist thinking: break any problem down into its component parts, solve the problem at the component level, and then reconstitute the whole to observe behavioural changes; reiterate this process as necessary until change in behaviour of the whole system of interest conforms to preset tolerance limits. Should the 21st century turn out to be the "biologists' century" then reductionism may turn out to be an unfortunate mindset in which to commence work. However, when investigating physiological systems and their pathologies, it is rare for more than one level of spacial scale to be considered as causal. This view needs to change in order to accelerate new approaches that combine precision medicine with personal traits.

For the purpose of this paper only multiple spacial scales are considered (cf. multiple time scale approaches) and its system of interest—cardiac morphogenesis—is considered together with pathological considerations surrounding a congenital heart disease termed the "tetralogy of Fallot".

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2. The physiological and pathophysiological context

The development of the human embryonic heart is still best described by the work of Srivastava and Olson (2000).[1] Before embryonic day 15 (E15) the heart structure is in the form of a tube, yet by day E32 this structure has morphed into a four-chambered heart that has recognizable features of its final shape and structure. It seems clearly that known processes are responsible for this cardiac morphogenesis and in most cases the development of the foetus continues normally. However in a small percentage of cases the heart is malformed as a consequence of signaling defects at this stage of its development. The types of defect include: elongation defect (i.e., parts of the heart are longer than they should be due to abnormal proliferation of cells); septation defect (i.e., there are issues with the inner wall that separates the left and right side of the heart); alignment defect (i.e., internal structures of the heart are not where they should be); and cushion defects (i.e., the heart valves and other internal structures have not developed properly). In turn these defects give rise to specific types of congenital heart disease; for example, common arterial trunk, double outlet right ventricle, and transposition of the great arteries. In this paper the tetralogy of Fallot is considered, which is defined as the association of four anatomic features: subpulmonary stenosis; an overriding aorta (less to the left than it should be); a ventricular septal defect (a "hole in the heart"); and right ventricle hypertrophy.

One of the key processes in cardiac morphogenesis occurs at the cellular level and is termed the epithelial to mesenchymal transition (EMT). It is the transformation of epithelial cells found in the inner wall of the primitive heart tube to mesenchymal cells that invade the extracellular matrix and give the tube some internal structure known as endocardial cushions. These cushions eventually develop into the internal heart valves and trabecular septa responsible for unidirectional blood flow within the heart (the former) and pumping efficiency (the latter). EMT simulation models are considered later in this paper.

3. A multiscale description of cardiac morphogenesis (E15 to E32)

3.1 The anatomic level

The structural development of the heart at all levels of spacial scale is more or less complete at day E32. The change in structure is immense. Figure 1 illustrates the changes in internal and external structures and conformations.

As early as the end of week 2 of gestation the two endocardial tubes that form the primitive heart fuse, giving rise to the first cardiac muscle contractions and blood circulation. At this stage the heart comprises an inner wall (endocardium) and outer wall (myocardium) with cardiac jelly (extracellular matrix) between them. The internal structure of the heart is formed at first by swellings at specific points in the walls of the heart tube (the endocardial cushions). This directed activity eventually divides the heart in two along its longitudinal axis, forming the left heart and right heart. At the same time each side of the heart is further divided into two, forming the atria and ventricles. By E32 the primitive heart tube has morphed into a four-chambered double pump, complete with orifices for the great arteries and veins to connect to. The external appearance of the heart also goes through key conformational changes between E24 and E32.

[1] D. Srivastava and E.N. Olson, A genetic blueprint for cardiac development, *Nature* **407** (2000) 221–226.

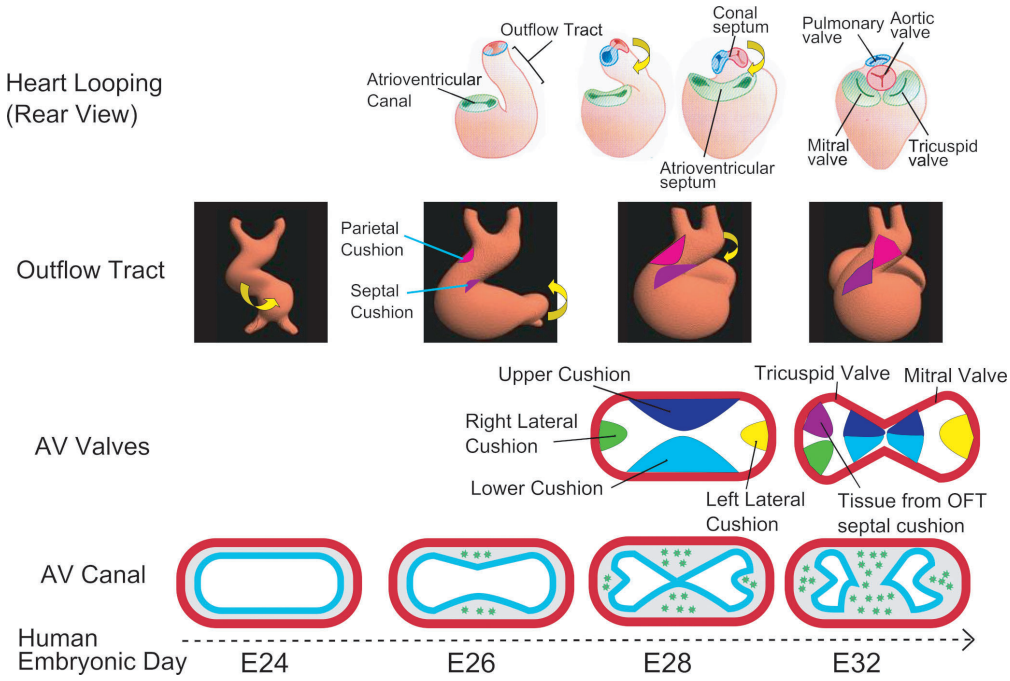

Figure 1. Cardiac morphogenesis at the anatomic level between E24 and E32.[2]

These changes are known as looping and wedging, and they occur concurrently. At roughly E26 the heart tube begins to loop on its right side into an S-shape. This is an important process as the conformational change brings all four heart chambers and their inflow and outflow orifices into the relative spacial positions found in the fully developed heart. Aortic wedging takes place as a consequence of the looping process, and ensures that this outflow tract is positioned correctly. Looping and wedging processes are both complete by about E28.

3.2 The cellular level

As stated above, EMT is an important process at the cellular level of cardiac morphogenesis, being responsible for the formation of the endocardial cushions. Figure 2 presents a simplistic overview of EMT. It illustrates schematically the inner and outer walls of the heart tube and the cardiac jelly (extracellular matrix) found between them. EMT is the process by which regularly shaped endocardial cells lose their adhesion to each other and invade the cardiac jelly. In doing so, the cells alter their morphology to become less rounded and more spiky. Their irregular shape, now adopting a mesenchymal phenotype, has consequences, especially increased adhesion in the cardiac jelly layer, allowing their concentration and volume to increase. This in turn leads to the formation of stable endocardial cushion structures that will eventually form the internal structures of the heart.

[2] T. Abdulla, R.A. Imms, J.-M. Schleich and R. Summers, Towards multi-scale systems modelling of epithelial to mesenchymal transition. In: *33rd Annual International Conference of the IEEE EMBS* (2011), pp. 449–452.

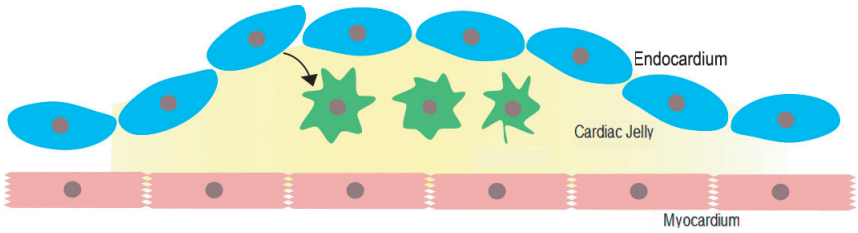

Figure 2. An overview of the epithelial to mesenchymal transition (EMT).[3]

3.3 The protein level

It is thought that most of the protein pathways responsible for EMT are known, although there is more work to do to determine exact functions and interactions between pathways. Areas in the cell wall where there is a loss of adhesion of endocardial cells correspond to a depletion of vascular endothelial cadherin proteins (e.g., the *VEGF* protein). *Notch* proteins also play an important role as they increase the expression of *Snail* transcription factors, and these inhibit the expression of *VEGF*, causing the drop in concentration that signals the commencement of EMT. In fact, although the proteins involved in the full analysis of cardiac morphogenesis form a network, there is evidence for a cybernetic description of their actions with both augmenting and inhibitory behaviours observed. For instance, *VEGF* protein concentration requires negative feedback control, since if that concentration is too high the endothelial phenotype is sustained and there is no cushion growth; if *VEGF* protein concentration is too low there are too few endocardial cells for prolonged EMT. However, within the "cybernetic control" window endothelial cells proliferate, which leads to enhanced cushion growth. The protein-level interactions responsible for cardiac morphogenesis are more complex than indicated here in terms of the number of component parts, the number of different protein pathways, and the number of pathway interactions. It is also becoming increasingly possible to consider molecular and genetic components that combine to initiate protein involvement.[4] Here, a further level of networks can be uncovered, again with a myriad of interconnexions. Systems theory formed from network analysis in other domains suggests that a simpler analysis may be possible, though the approach currently eludes researchers in the field.

4. Aspects of modeling

The use of models in cardiac morphogenesis to help represent component parts has proved to be an effective way of understanding and explaining real-world behaviour. Here, two types of model are considered: *information models* based on an ontological approach, which may be one way to link together models constructed at different spacial scales; and *simulation models* that provide a more direct approach of understanding behaviour, in this case of EMT as an example of what can be achieved.

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[3] R. Summers, T. Abdulla and J.-M. Schleich, Progress with multi-scale systems, *Measurement Control* **44** (2011) 180–185.

[4] M.S. Rana, V.M. Christoffels and A.F.M. Moorman, A molecular and genetic outline of cardiac morphogenesis, *Acta Physiologica* **207** (2013) 588–615.

4.1 Information models

Methods of representing processes involved in cardiac morphogenesis (more specifically, EMT) can be seen in Figure 3, which also indicates the approximate levels of spacial and temporal scales deployed in the representations, exemplary markup languages available, and the published ontologies that can be used to link models together (a glossary at the end of this paper defines the acronyms used). For the purpose of this paper an ontology can be defined as "a set of concepts and categories belonging to cardiac morphogenesis that shows their properties and the relations between them". The XML markup language forces a declarative expression of model components that enables them to be interpreted by different modeling platforms. This is the key to how scale linking can occur. Annotation of XML expressions can create an explicit link between actors in the model and external identifiers that can be interpreted directly by software agents. The ontologies available at different levels of spacial scale are shown and by using information modeling conventions they can be classified as "occurents", "independent continuants", and "dependent continuants". By making these distinctions a clear boundary can be drawn between the spacial and temporal domains.

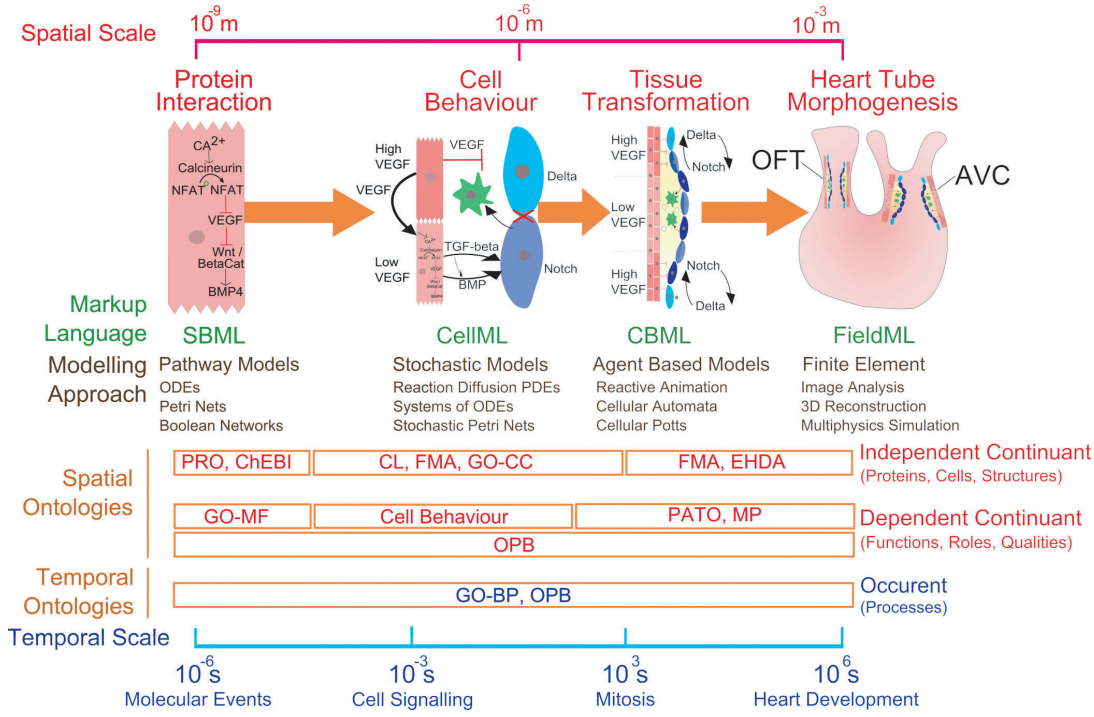

Figure 3. Representation of the epithelial to mesenchymal transition (EMT), the spacial and temporal scales involved, and the published ontologies applicable. [5]

[5] T. Abdulla, R.A. Imms, J.-M. Schleich and R. Summers, Towards composite annotation for heart development. In: *International Conference on Biomedical Ontology* (2011), pp. 47–54.

As simulation models comprise both spacial and temporal components it becomes necessary to combine terms using a post-composition approach, which makes use of the entity quality (EQ) formalism that links terms from a given ontology in use to a quality term in the phenotype and trait ontology (PATO). A key application of this approach is the integration of phenotypic annotations from multiple species. As one might expect, investigation of human cardiac morphogenesis makes extensive use of murine and chick models together with the use of zebrafish; being able to annotate processes across species aids understanding of concepts in human cardiac development. The post-composition approach has also been used for the annotation of simulation models, though using the ontology of physics and biology (OPB) rather than PATO—as simulations tend to represent the physical properties of biological entities such as concentrations or densities. Hence, whereas PATO allows composite phenotype annotations such as "endocardial cushion with decreased level of *snail*", the OPB allows the representation of the same composite term but with reference to (say) the measured concentration of a protein. Should the protein concentration be a parameter in the simulation model, the annotation provides the link between the information model and the physical simulation.

4.2 Simulation models

An *in vitro* study of explant murine endocardial cells cultured on a supporting collagen gel demonstrated the actions of *Notch1* and *BMP2* in EMT.[6] Figure 4 shows the morphological changes in cells quite clearly, together with invasion of the supporting gel caused by changes in cell adhesion. In the *in vivo* state, the invasion of the gel implies endocardial cushion formation. To simulate these behaviours *in silico*,[7] cellular Potts models (CPMs) were used.[8] CPMs are lattice-based simulations of cell populations whose behaviours are driven by a generalized Hamiltonian energy, *H*. More motile cells tend to reduce *H*. The model environment includes terms to model interactions between adjacent lattice cells and between lattice cells and their simulated supporting gel—representing cell adhesion. CPMs include constraints on cell volumes and surface areas, thus useful for modeling change of cell morphology in EMT. The spacial environment surrounding the simulated culture is taken as *H*-inert; that is, it has no interaction with the Hamiltonian system—indicated by it being given the value zero in Table 1 below.

For the simulation whose results are shown in Figure 5, the Compucell3D simulation environment was chosen, which uses a Monte Carlo algorithm to derive simulated behavioural changes at each of its steps. During each one a copy attempt is made from each lattice cell to one of its six neighbours picked at random. The copy is accepted or rejected using a probability function that is itself influenced by the overall goal to maintain *H*. Table 1 shows the surface energy parameters used to demonstrate the simulated behaviours shown in Figure 5, where an initial layout of 100 cells arranged in a circular monolayer were simulated for 1000 steps.

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[6] L. Luna-Zurita, B. Prados, J. Grego-Bessa et al., Integration of a Notch-dependent mesenchymal gene program and BMP2-driven cell invasiveness regulates murine cardiac valve formation, *Journal of Clinical Investigation* **120** (2010) 3493–3507.

[7] That is, with a digital computer.

[8] T. Abdulla, R. Imms, J-L. Dillenseger, J.-M. Schleich and R. Summers, Computational modelling of epithelial to mesenchymal transition, *IRBM* **32** (2011) 306–310.

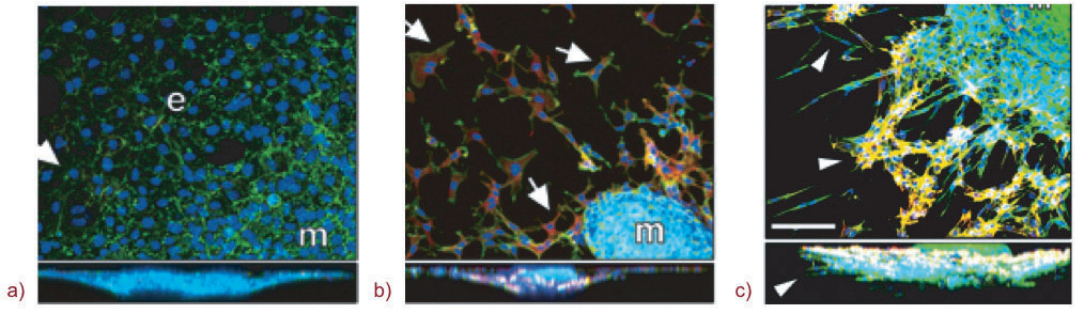

Figure 4. *In vitro* endocardial explants: a) original tissue remains in a monolayer; cells are circular in shape; b) Notch protein-activated cells scatter on the surface, change in cell morphology, some cell invasion of the supporting collagen matrix; c) with further addition of BMP2 protein, change in cell shape are clearly seen, and increased invasion of the supporting matrix, forming endocardial cushions.

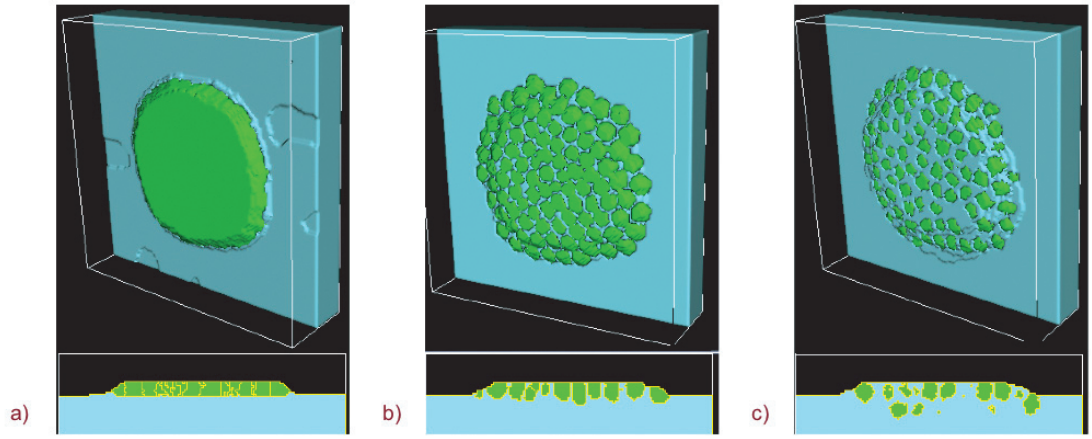

Figure 5. Simulations of *in vitro* EMT: a) endocardial monolayer remains on the surface of the supporting gel and cell shape is circular; b) simulation of reduced endocardial cell-to-endocardial cell adhesion: cells scatter and, change shape but there is no invasion of the supporting gel; c) simulation as in b) with increased endocardial cell-to-gel adhesion, some irregularly shaped cells, and invasion of the supporting gel.

Table 1. Surface energy parameters.

	EC–env	**Gel–env**	**Gel–Gel**	**EC–Gel**	**EC–EC**	**env–env**
Set 1	16	14	8	4	2	0
Set 2	16	14	8	4	10	0
Set 3	16	14	8	1	10	0

From the simulations using the parameters in Table 1, Set 1 gave rise to an endocardial monolayer (cf. Figures 4a and 5a); Set 2 corresponds to a loss of endocardial cell cohesion (cf. Figures 4b and 5b); and Set 3 simulates both the loss of endocardial cell cohesion and an increase in endocardial cell-to-gel adhesion (cf. Figures 4c and 5c).

In each scenario the *in silico* simulation mimicked results from the *in vitro* study sufficient well to satisfy the purpose of the study. Both approaches aid the understanding of how proteins, their pathways and their signals influence EMT.

5. Discussion

This paper considers some of the issues that surround the process of developing a multiscale system framework applied to specific physiological phenomena. Though the majority of current work is targeted at adult physiology, the motivation of the present line of investigation that the challenges involved in representing cardiac morphogenesis would be a real test of methods employed and software tools deployed. As predicted there are gaps not only in knowledge and understanding of physiological processes, they are also found in the methods and tools used to study them. However, progress has been made, methods and tools have been adapted where necessary, and results disseminated.

It is clear from the published literature that the majority of models in computational biology are limited to a single spacial scale; biological systems, however, are inherently multiscale. One of the engineering drivers of the work discussed here is to reduce the mismatch in approach between "real world" and "model world" by using applied systems thinking. A factor that is often overlooked in conventional model representations is the ability to re-use components from previously published models. In multiscale models of physiological processes the modeling community can draw upon an international resource—the IUPS Physiome project. This project comprises a curated repository of multiscale models of various physiological systems, held at the University of Auckland, New Zealand. Using this resource, re-use of model components is commonplace; for example, multiscale models of cardiac electrophysiology have re-used models of calcium dynamics taken from another source.[9] A key requirement for re-use of model components is annotation, as outlined in this paper. Minimum information guidelines for the annotation of models[10] and simulations[11] have been suggested, which will help alleviate the problem of inconsistency of descriptors used between annotators. The use of an XML-based declarative approach is extremely useful for making annotations but it is also possible to create an explicit link between model entities and external, web-accessible resources—perhaps to uniform resource identifiers. At this point a personal plea can be made—given the haphazard naming conventions for proteins, can a modern-day Linnaeus be found to classify and categorize proteins in existing ontologies to enable more generic annotations of unique identifiers?

The complexities involved in modeling EMT have also been shown in outline above. In the simulation shown information was used from cell and protein levels of spacial scale; gene-to-phenotype associations are becoming increasingly accessible. This gives hope that genetic-level

[9] P.J. Hunter, E.J. Crampin and P.M.F. Nielsen, Bioinformatics, multi-scale modelling and the IUPS Physiome project, *Briefings in Bioinformatics* **9** (2008) 333–343.

[10] N. Juty, N. Le Novere, C. Laibe, Identifiers.org and MIRIAM registry: community resources to provide persistent identification, *Nucleic Acids Research* **40** (2012) D580–D586.

[11] D. Waltemath, R. Adams, D.A. Beard et al., Minimum information about a simulation experiment (MAISE). *PLoS Computational Biology* **7** (2011) e1001122.

interventions can be utilized in the future to combat congenital heart disease. But there are hidden complexities too; it is not always true that one particular gene is responsible for one specific disease. The reality is that a network of genes is implicated in more than one physiological mechanism, which can lead to one of several pathologies.

6. Conclusions

This paper has outlined the role of information models and simulation which, when taken together, increase the multiscale understanding of cardiac morphogenesis in general, and EMT specifically. Protein-level interactions involved in EMT remain highly complex and are still only partly understood. A multiscale model may be able to pinpoint gaps in knowledge and understanding and highlight missing pieces in the ontological jigsaw, not just in the application under consideration but also on any occasion where a component is re-used. For example, a multiscale model of the *Delta–Notch* protein signaling network is highly likely to provide a re-usable model component that can slot into many other multiscale models and simulations, given the widespread nature of its involvement in other physiological processes.

The work described in this paper now continues in two separate strands. Scale linking is needed for continued success of the multiscale approach and will increase the utility of models, simulations and their re-usable components. Though work elsewhere is investigating a software platform to integrate spacial scales (for example, see the IUPS Physiome project[12]), the approach taken here remains with an underpinning information model. The second strand makes use of a national (French) resource of cadaver hearts stored at the Marie Lannelongue Hospital, Paris. Using the subset that comprises cadaver hearts diagnosed with tetralogy of Fallot, colleagues are redefining the anatomical landmarks of the condition via magnetic resonance imaging and computed tomography. By doing so, more precise measurements can be determined of the degree of rotation of the outflow tract (overriding aorta), from which further anatomic landmarks can be derived.

Acknowledgment

I must recognize the influences of my former team in the writing of this article, especially Dr Tariq Abdulla and Prof. Jean-Marc Schleich. To them go the plaudits, though any errors in this paper are mine alone.

Appendix: Glossary of acronyms

Schematic diagram

AVC	Atrioventricular canal
BMP/BMP4	Bone morphogenetic protein family
DELTA	Protein—part of *Delta–Notch* protein signaling network
NFAT	Protein family—nuclear factor of activated T-cells
NOTCH	Protein—part of *Delta–Notch* protein signaling network
OFT	Outflow tract

[12] The Physiome project, see www.physiome.org (accessed July 27 2018).

TGF-β	Protein family—transforming growth factor
VEGF	Protein—vascular endothelial growth factor
Wnt	Protein family
Markup languages	
CBML	Cell behaviour markup language
CellML	Cell markup language
FieldML	Field markup language
SBML	System biology markup language
Ontologies—independent continuant	
ChEBI	Chemical entities of biological interest
CL	Cell type ontology
EHDA	Edinburgh human developmental anatomy
FMA	Foundational model of anatomy
GO–CC	Gene ontology–cellular component
PRO	Protein ontology
Ontologies—dependent continuant	
GO–MF	Gene ontology–molecular function
MP	Mammalian phenotype
OPB	Ontology of physics for biology
PATO	Phenotype and trait ontology
Ontologies—Occurent	
GO–BP	Gene ontology–biological process.