Morphology, growth and patterning of the developing heart: methods and applications

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Scope
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Congenital cardiac malformations are among the leading birth defects in human live births, affecting 1 in 100 children (Hoffman et al., 2004; Hoffman and Christianson, 1978; Marelli et al., 2007). This high incidence of structural heart defects highlights the complexity of cardiac development and, indeed, heart development was shown to depend on an interplay of a large number of genes at different stages of development and in different compartments of the heart (Bruneau, 2002; Franco et al., 1998; Gruber and Epstein, 2004).

Normal and abnormal heart development is studied using animal models. In this thesis both chicken and mouse embryos were used. Chicken embryos have two main advantages: Firstly, early heart development is relatively easy to understand because the different phases, namely formation of the tube, the tubular phase and chamber formation, are clearly separated in time. Secondly, chicken embryos are easily accessible which makes interventions relatively easy. On the other hand, the mouse is genetically much closer related to human, which makes the mouse heart a more suitable model for research on the genetic disorders that cause heart defects. Moreover, the required tools for genetic modifications and lineage analysis are readily available in mouse.

Heart development takes place at a terrific pace. In mouse (Fig. 1) the two laterally extending myocardialized channels that are present at embryonic day 7 (E7) fold together within hours to form a closed primary heart tube at E8. The now beating heart consists only of a ballooning primitive ventricle and an outflow tract. Only two days later, at E10, this continuously beating heart tube shows clear compartments, although still organized in tubular fashion: Atria start to balloon and the still undivided ventricles start to trabeculate. At E12.5, septation of the chambers is in an advanced state. Near the end of gestation, at E17.5, the heart is completely organised as it is in the adult.

![3D Reconstructions of the developing mouse heart (embryonic day 7 through 17.5)](image)

**Figure 1** 3D Reconstructions of the developing mouse heart (embryonic day 7 through 17.5)

To enable this transformation, the heart has to be a highly dynamic structure; cells are added continuously at both poles (Cai et al., 2003; van den Berg et al., 2009), while concomitantly cells from the outflow tract move into the right ventricle (Rana et al., 2007) and cells from the atrioventricular canal into the left ventricle (Aanhaanen et al., 2009). Local differences in proliferation rate are another important parameter in heart morphogenesis. The ballooning chambers show for instance higher proliferation rates than non-chamber myocardium (Soufan et al., 2006).
These rapid changes in shape of the heart are the result of interplay between gene expression and local growth. To comprehend the processes involved, we need tools to understand morphology, to measure and visualize morphogenetic parameters and to interpret associations in gene expression patterns. Although enormous amounts of gene expression data are available in literature, the exploration of this wealth of information is hampered by the fact that each paper reports the expression patterns of only a limited number of genes in a limited number of sections, rather than the entire heart. To remedy this situation, several initiatives have attempted to bring these data together in spatio-temporal gene expression atlases.

In Chapter 1, we reviewed such gene expression atlases to provide insights into both the possibilities of the atlases, as well as to describe what more than a decade of developmental gene expression atlases can teach us about the requirements of the design of the ‘ideal atlas’. One of the conclusions of this chapter is that the ‘ideal atlas’ should be based on a spatial framework.

The development of a program for TRacing the Anatomical Context of Tissue Sections (TRACTS) was based on such a framework. The aim of this program was twofold. Firstly, to place individual sections into a 3D reference model to be of help to the non-morphological scientist in need of a histological confirmation of a gene expression profile. Placing sections in a cardiac reference model helps in the exact annotation of the cardiac compartments present in the section. This may solve much of the disagreement among embryologists which may merely be based on miscommunication, because it is far from easy to form a mental image of a dynamically changing three-dimensional structure, even for experts (de Boer et al., 2007). Secondly, TRACTS can be used to collect the gene expression patterns visible in the fitted sections within one common spatial framework. The initial performance of TRACTS, i.e. how well TRACTS fitted a section, was judged by experts in morphology (Chapter 2). In this version of TRACTS, the reference model was reconstructed from histological sections stained with myocardium-specific markers (Soufan et al., 2003).

In the final version of TRACTS, the spatial framework was improved by using a reconstruction based on episcopic images which are not subject to deformations due to sectioning and stretching (Weninger et al., 2006; Weninger and Mohun, 2002). Now the performance of TRACTS was compared to that of experts in morphology (Chapter 3). This chapter also describes how 3D reconstructions can easily be disseminated using interactive 3D-pdf’s. These allow the manipulation of the 3D structure on one’s own computer screen which is ideal for the understanding of the intricate morphology of developing embryos.

In Chapter 4, we describe and validate a tool which was used to measure the speed at which cells migrate from the growth center in the caudal pericardial wall into the non-proliferating primary heart tube. The results showed that these cells migrate at a speed of 70 µm per hour (van den Berg et al., 2009).

The development of the mouse heart from the earliest signs of the formation of the heart to the end of the fetal period is described in Chapter 5. This description is based on morphological and quantitative 3D reconstructions. The latter rely on BrdU-labelling experiments. BrdU is a thymidine analogue which is incorporated during DNA synthesis which occurs in the S-phase of the cell cycle. This study resulted in a comprehensive series of interactive 3D reconstructions showing the mouse heart development throughout the gestational period, each supplemented by the pattern of proliferation. A detailed analysis of BrdU-positive fractions showed that trabeculation of the cardiac chambers occurs from a focus of proliferation at the base of each trabecule.
Although the BrdU-labelling indices are related to the length of the cell cycle, a direct interpretation is hampered by the relative length of the S-phase compared to the total cell cycle length. Therefore, in Chapter 6, we describe a method to measure the actual local cell cycle lengths by labelling for two different exposure times with two thymidine analogues, namely IdU and CldU. This method was applied on early embryonic chicken hearts and showed an extremely heterogeneous pattern of cell cycle lengths, with lengths ranging from less than 8 hours in the growth centers in the caudal pericardial wall, less than 16 hours in the forming primitive ventricle, to days in the primary myocardium.

As was described in Chapter 1, anatomical annotation is often controversial because of inconsistent anatomical nomenclature and definition of borders. These controversies can be, at least partially, solved by using annotated reference models. Since the meaning of terms used in anatomically annotated reference models will be clarified by their 3D spatial depiction, it will be immediately evident what the defined borders of named structures are. This will remove most of the ambiguity in the terms used in anatomical annotations. A complicating aspect of using anatomical annotation is that gene expression patterns do not respect borders of anatomical structures (Ruijter et al., 2004).

To address the latter problem it was proposed in Chapter 1 to combine the anatomical annotation of a spatial framework with an annotation based on the expression profiles of a limited set of genes with known expression domains. To this end we mapped the expression patterns of 12 genes involved in heart development to the previously published reference heart (Chapter 7). We applied hierarchical clustering to all locations in the heart based on those expression patterns. Dynamic pruning of the dendrogram resulted in 18 continuous domains, each with its own unique expression profile. Most of these domains represented classical anatomical components, but we also observed novel domains that cannot be distinguished by any anatomical landmark.

The presented methods are tools to define and communicate on the anatomy and morphometrics of the developing heart. Application of the described methods provided new findings and biological insights into the morphogenesis of the heart. Our tools will enable the integration of quantitative morphological and gene expression data within a single spatial framework, which will in turn allow a systems biological approach of cardiac development.

References


