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Review

Xenopus as a model system for vertebrate heart development

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Abstract

The African clawed frog, *Xenopus laevis*, is a valuable model system for studies of vertebrate heart development. In the following review, we describe a range of embryological and molecular methodologies that are used in *Xenopus* research and discuss key discoveries relating to heart development that have been made using this model system. We also discuss how the sequence of the *Xenopus tropicalis* genome provides a valuable tool for identification of orthologous genes and for identification of evolutionarily conserved promoter elements. Finally, both forward and reverse genetic approaches are currently being applied to *Xenopus* for the study of vertebrate heart development.

Keywords: Explants; Mis-expression; Antisense oligonucleotides; Transgenic animals; Promoter analysis

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1. Introduction

Heart development is a beautifully orchestrated process that is highly conserved in all vertebrate organisms. This fact is most clearly illustrated by the observation that the heart of all vertebrates is practically indistinguishable through the linear heart tube stage and into the early stages of looping morphogenesis and chamber formation. This conservation has allowed researchers in the field of vertebrate heart development to utilize numerous animal models including zebrafish, Amphibia, chicken and rodents to further our understanding of the molecular and morphological aspects of human heart development and congenital heart disease. While researchers might argue the advantages of their particular model system, it is important to realize that the complementary experimental approaches offered by different systems has greatly increased our rate of progress in understanding the overall process of cardiogenesis. Here we will review a series of studies illustrating the ways in which the frog, *Xenopus laevis* and the related species *Xenopus tropicalis*,

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Table 1
Timing of significant stages of heart development in *Xenopus laevis* (adapted from [66] with permission from Wiley)

Morphological event	Stage of development [67]	Hours post-fertilization at room temperature
Specification	12–14	15–17
Migration to heart progenitors to ventral midline	26–28	30–32
Heart tube formation	31–33	32–35
Coordinated muscle contraction	35	46
Looping	33–36	44–50
Chamber differentiation	39–40	60
Valve formation	41–44	70–90
Atrial septation	44–45	92
Mature heart	46	106

have been used to further our understanding of vertebrate heart development. In addition, we will discuss recent resources and methodologies that have been made possible by the genomic revolution and which further increase the usefulness of *Xenopus* as an animal model.

Studies of heart development in the early 20th Century typically used frog and Amphibian species other than *Xenopus*. Amphibia had become a favorite model system of embryologists due to the large size of the eggs, the large number of embryos, which develop outside of the mother and the fact that the embryos exhibit a remarkable ability to heal after microsurgery. In the past, these studies where often hindered by their dependence on wild-caught embryos. The observation in the 1930s that *Xenopus* could be easily and reliably induced to ovulate large numbers of eggs year round [1,2] combined with the fact that they could be raised and housed easily within the lab made this species of frog a favorite for many developmental biologists around the world.

2. Heart development in *Xenopus*

Through a combination of lineage tracing [3–5] and transplant experiments [6,7], the following picture of the earliest events in normal *Xenopus* heart development has emerged. A timeline of significant steps of *Xenopus* heart development is outlined in Table 1. The heart, which ultimately occupies a ventral position within the chest of the mature tadpole/frog, starts out as two bilateral patches of specified mesoderm on the dorsal side of the embryo at the onset of gastrulation. Gastrulation movements cause the heart patches to move dorso-anteriorly until, during neurulation, the heart progenitors begin to migrate ventrally where they meet in the anterior region of the embryo at the ventral midline. Expression of numerous RNA and protein markers of the cardiac muscle lineage is first detected at the bilateral heart patch stage prior to fusion of the heart primordia. At the ventral midline the two heart patches fuse to form a simple linear tube, before undergoing the looping and remodeling processes of cardiac morphogenesis.

The availability of molecular markers has greatly facilitated detailed analysis of the morphological events involved in development of the mature frog heart. This has been achieved through in situ hybridization of cardiac markers to histological sections followed by 3D reconstruction [8] and also by immunohistological detection of heart tissue followed by whole mount confocal microscopy [9]. Through use of these methods we now have an excellent description of *Xenopus* heart development from the heart patch stage to chamber formation. In general the events of frog heart development closely resemble the equivalent processes of heart development in higher vertebrates including a leftward bend of the outflow tract, the presence of an atrioventricular valve to separate the atria and ventricle, asymmetric division of the atria early in development (with the right side being larger), and the presence of trabeculae within the thickened wall of the ventricular myocardium. Of course some differences do exist and *Xenopus* ultimately possesses a three-chambered heart (one ventricle and two atria) rather than the four-chambered heart of birds and mammals. These differences in the *Xenopus* heart need not be viewed as a hindrance but may present valuable opportunities for investigation of the molecular mechanisms underlying formation of a heart that is an evolutionary intermediate between the two-chambered heart of fish and the four-chambered heart of birds and mammals.

3. Methods for studying heart development in *Xenopus*

3.1. Microinjections

The large size of the *Xenopus* embryo and its development outside of the mother make *Xenopus* extremely well suited for manipulations of gene activity via microinjection. Typically microinjection studies are used to examine the function of a gene of interest by overexpression of the wild type or mutant sequence or by loss of function approaches. Furthermore, the extremely reproducible cleavage patterns of the frog embryo (detailed by fate mapping studies [3–5]) facilitate targeting of injected material to restricted lineages of the developing embryo.

3.1.1. Overexpression/mis-expression

Overexpression/mis-expression studies in *Xenopus* are easily achieved by microinjection of in vitro synthesized mRNA, or in some cases plasmid DNA [10], into the single cell *Xenopus* egg before the first cleavage or into selected blastomeres. Injection of mRNA into a fertilized *Xenopus* egg prior to first cleavage generally results in global overexpression because the injected mRNA diffuses fairly broadly. Since the plane of first cleavage typically separates the left and right sides of the embryo, microinjection into one cell of a two-cell embryo results in overexpression on one side of the embryo only. One-sided injection is particularly useful for studies of early heart development, prior to fusion of the heart patches, because the uninjected side of the embryo serves as a stage-matched control for comparison. Injections after the two-cell stage (typically 8 and 16 cell embryos) can also be useful because the injected mRNA can be targeted so that it is overexpressed preferentially in the cardiac lineage. An example of the usefulness of this type of experiment is provided by investigations into the role of the homeodomain transcription factor Nkx2–5 in heart development. Nkx2–5 is the vertebrate