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Review

Frogs as integrative models for understanding digestive organ development and evolution

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ABSTRACT

The digestive system comprises numerous cells, tissues and organs that are essential for the proper assimilation of nutrients and energy. Many aspects of digestive organ function are highly conserved among vertebrates, yet the final anatomical configuration of the gut varies widely between species, especially those with different diets. Improved understanding of the complex molecular and cellular events that orchestrate digestive organ development is pertinent to many areas of biology and medicine, including the regeneration or replacement of diseased organs, the etiology of digestive organ birth defects, and the evolution of specialized features of digestive anatomy. In this review, we highlight specific examples of how investigations using *Xenopus laevis* frog embryos have revealed insight into the molecular and cellular dynamics of digestive organ patterning and morphogenesis that would have been difficult to obtain in other animal models. Additionally, we discuss recent studies of gut development in non-model frog species with unique feeding strategies, such as *Lepidobatrachus laevis* and *Eleutherodactylous coqui*, which are beginning to provide glimpses of the evolutionary mechanisms that may generate morphological variation in the digestive tract. The unparalleled experimental versatility of frog embryos make them excellent, integrative models for studying digestive organ development across multiple disciplines.

Contents

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1. Introduction

The anatomical and physiological complexity of the vertebrate digestive system develops from a simple primitive gut tube (PGT). This PGT undergoes intricate patterning and differentiation events to enable the epithelial lining of the tube to assume the absorptive and secretory functions required of a gastrointestinal (GI) tract, while discrete segments bud off of the original structure to form accessory organs, including the pancreas and liver. Concomitantly, the tube lengthens and rotates, as it transforms from a short, occluded cylinder to a long, hollow conduit arranged in a three dimensional configuration of loops and coils.

Elucidating the mechanisms of digestive organ development has broad implications for many areas of biology and medicine. Some of the most common human birth defects affect the digestive tract, yet the genetic and/or environmental factors that contribute to the etiology of these malformations remain to be discovered. In addition, diseases of the digestive system affect millions worldwide, generating substantial demand for therapeutic interventions; full knowledge of the developmental events that pattern and shape the PGT is likely to be vital for successful regeneration or engineering of human digestive tissues. Finally, although many features of digestive anatomy are highly conserved among vertebrates, the length, compartmentalization and topological orientation of the GI tract can vary tremendously among and between species, especially those with different diets, yet the evolutionary origins of this ecologically-relevant variation are largely unknown.

1.1. The advantages of the frog embryo

Amphibians have long been used as model organisms for studying embryonic development, and have played instrumental roles in unraveling the intricate events that guide germ layer formation, gastrulation and neurulation [1–3]. Beyond early development, frog embryos also boast several advantages for the study of organ specification and morphogenesis [4]. Unlike amniote embryos that are confined to a uterus or shell during development, frog embryos are externally fertilized and can be easily cultured *in vitro*, making them amenable to a wide variety of experimental manipulations. For example, the rate of development of frog embryos can be accelerated or slowed by adjusting temperature, facilitating convenient analyses of any stage of organogenesis [5]. Moreover, precise fate maps have been generated for the early blastomeres (32-cell stage), allowing loss- and/or gain-of-function (LOF/GOF) reagents and lineage tracers to be targeted to specific organs by standard microinjection technology, enabling gene function to be queried in a tissue-specific manner [6,7]. Furthermore, because frog embryos are relatively large and harbor an innate, intracellular yolk supply, tissue explants can be dissected, recombined and transplanted, or cultured in simple saline, at almost any stage of development, facilitating expedient, inexpensive specification and trans-differentiation studies [8–14]. Finally, the frog embryo's accessibility to chemical agonists/antagonists allows the role of specific signaling pathways to be interrogated during critical windows of organogenesis (*i.e.*, subsequent to earlier developmental events that may also depend on such pathways). In fact, thanks to large clutch sizes, frog embryos provide a powerful platform for high-throughput "chemical genetic" or toxin screening using organ morphology as a phenotypic readout [15–18]. This experimental amenability makes the frog embryo an ideal model in which to interrogate the mechanisms of organ development.

1.2. More than one frog in the water

Amphibian models (mainly urodeles) have been employed in developmental biology research for over a century, but the convenience of *in vitro* fertilization methods made *Xenopus* species the most popular frogs in the laboratory [19]. Nonetheless, many non-model frog species are equally amenable to experimentation as *Xenopus*. Comparative "evo-devo" studies utilizing frogs with different reproductive strategies and/or developmental rates [20] are beginning to provide fascinating insight into the molecular and cellular mechanisms that shape different embryos, while species that fill unique ecological niches or possess intriguing specializations are shedding light on the developmental origins of novel morphologies [21].

In this review, we provide a broad perspective on the ways in which *Xenopus* and emerging frog models have yielded new insight into digestive organ patterning, morphogenesis, and evolution.

2. What can the frog tell us about foregut organ specification?

The developing digestive tract may be divided into foregut (esophagus, stomach, duodenum, liver, pancreas, gall bladder) and midgut/hindgut (intestine) domains. The foregut-derived organs play critical roles in processes such as digestion, glucose homeostasis, and detoxification. Therefore, congenital defects or disease in these organs (*e.g.*, diabetes, pancreatitis, fatty liver disease, biliary atresia, gall stones and gastric/pancreatic cancer) are the cause of substantial morbidity and mortality worldwide [22–25]. To ameliorate such afflictions, translational researchers seek to develop regenerative therapies and engineer replacement tissues *in vitro*. Progress in these areas has been profoundly influenced by models of the normal process of foregut organ specification and morphogenesis in the embryo [26,27].

In all vertebrates, the PGT is comprised of an inner endoderm layer, which differentiates into the epithelial lining of the GI tract, surrounded by an outer layer of mesoderm, which will give rise to the visceral muscle and connective tissue. Early in gut development, reciprocal signaling between the endoderm and mesoderm layers gradually distinguishes anterior foregut and posterior hindgut domains [28]. In addition to digestive tissues, numerous structures with diverse physiological functions are derived from the