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Engineering *Xenopus* embryos for phenotypic drug discovery screening ☆

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ABSTRACT

Many rare human inherited diseases remain untreatable despite the fact that the disease causing genes are known and adequate mouse disease models have been developed. In vivo phenotypic drug screening relies on isolating drug candidates by their ability to produce a desired therapeutic phenotype in whole organisms. Embryos of zebrafish and *Xenopus* frogs are abundant, small and free-living. They can be easily arrayed in multi-well dishes and treated with small organic molecules. With the development of novel genome modification tools, such a zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas, it is now possible to efficiently engineer non-mammalian models of inherited human diseases. Here, we will review the rapid progress made in adapting these novel genome editing tools to *Xenopus*. The advantages of *Xenopus* embryos as in vivo models to study human inherited diseases will be presented and their utility for drug discovery screening will be discussed. Being a tetrapod, *Xenopus* complements zebrafish as an indispensable non-mammalian animal model for the study of human disease pathologies and the discovery of novel therapeutics for inherited diseases.

Contents

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1. Introduction

Rare diseases, also referred to as orphan diseases, are classified as diseases that affect a small percentage of the human population [1]. A rare disease has been defined such as one with a prevalence of less than 1 in 2000 and those with 1 in 50,000 are considered ultra-rare [2]. Most rare diseases have a genetic basis, and thus they are present throughout the patient's life. Disease onset may vary and symptoms do not have to appear immediately. Nevertheless, children are particularly affected and about 30% of the affected children will die before reaching their fifth birthday. Rare inherited diseases can vary in prevalence between populations. A disease that is rare in the general population may be common among members of specific ethnic groups. For the European Union, rare inherited diseases are estimated to affect as much as 6–8% of the population, and worldwide the numbers are in the range of 350 million people. Despite the fact that many rare inherited diseases are of life threatening and/or chronically debilitating nature, no or only inadequate treatment options are available. Enzyme replacement for Gaucher's disease, bone marrow transplantation for some forms of leukemia, and gene therapy for rare immune deficiency disorders represent exceptions to this rule [3].

Target-based drug discovery, the standard approach practiced by the pharmaceutical industry for the last 30 years, has mostly failed to address the needs of patients suffering from inherited rare diseases. Phenotypic drug screening has recently been shown to be more efficacious than *target-based* approaches in the discovery of first-in-class small-molecule drugs [4]. Phenotypic drug discovery relies on screening intact cells or whole organisms with chemical libraries of synthetic small organic molecules, natural products or extracts to identify substances that have a therapeutic effect [5–7]. Therefore, phenotypic drug discovery represents a novel, promising approach to meet the therapeutic needs of patients with inherited diseases.

In vivo phenotypic drug screening uses model organisms to identify novel bioactive compounds that could not be recovered with standard in vitro approaches relying on cell culture systems. Seeded on flat culture dishes covered by simplified extracellular matrices and supplemented by artificial culture media, cells used in vitro are no longer in their natural context of the body. If cell lines are used in place of primary cells, they have undergone profound genetic and epigenetic changes in the process leading to immortalization. By contrast, the cells of an intact organism are non-transformed and found in their normal context within organs and tissues, where they are exposed to cell–cell and cell–matrix interactions in a three-dimensional context. Certain drug candidates may require biotransformation in the liver to become active as a metabolite. Such compounds are expected to score negative, if tested in cell culture systems. Drug candidates discovered by their ability to elicit a specific therapeutic effect in an animal model are also likely to fulfill the efficacy and specificity requirements that need to be met by promising therapeutic agents earmarked to enter clinical development. These include proven efficacy, good cell permeability, lack of obvious toxicities, and favorable pharmacodynamic and pharmacokinetic profiles. In vivo phenotypic drug screening, therefore, combines screening and animal testing in one step.

Over the last ten years, embryos of zebrafish (*Danio rerio*) have been very successfully used as whole-organism in vivo bioassay systems to identify novel bioactive compounds with first examples entering clinical testing [8]. Limitations in translating directly from zebrafish to mammalian systems however exist, as demonstrated best by the example of persynthamide, a promising small organic molecule with cell cycle modulating activities. Persynthamide was identified in a screen of a 16,000-compound library for synthetic organic molecules that suppressed the mitotic phenotype observed in the recessive zebrafish cell cycle mutant *crash&burn* (*crb*) [9]. The *crb* mutation affects *mybl2* (formerly known as *bmyb*) gene and causes an increase in the number of mitotic cells in the embryo [10]. Homozygous *crb* mutant zebrafish are viable and thus can be employed for drug screening purposes. Persynthamide was recovered as the only molecule from the chemical library screen able to rescue the mitotic and apoptotic phenotypes observed in homozygous *crb* mutant zebrafish embryos. It was considered a promising antitumor agent because of its ability to suppress the cell cycle defect in *crb* mutant zebrafish without affecting wild-type embryos [9]. The effects of persynthamide on cell cycle regulation were however found to be zebrafish-specific and could not be generalized to

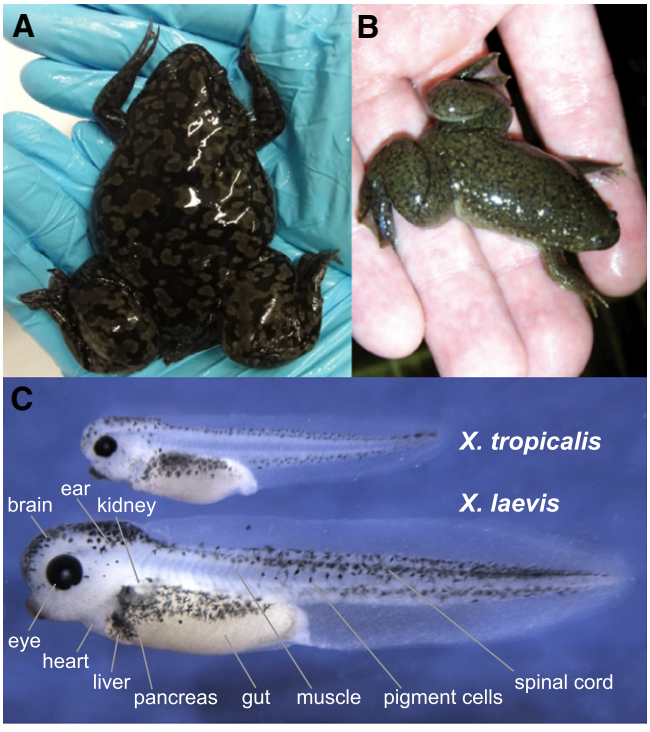

Fig. 1. *Xenopus* frogs and tadpoles. A, B) Comparison of adult female *X. laevis* (A) and *X. tropicalis* (B) frogs. C) *Xenopus* embryos at stage 41 (3 days post fertilization). Positions of the key organs of the vertebrate body plan are indicated. At this stage, they have begun to execute their dedicated physiological functions essential for survival of the embryo.