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Review

Using *Xenopus laevis* retinal and spinal neurons to study mechanisms of axon guidance *in vivo* and *in vitro*

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ABSTRACT

The intricate and precise establishment of neuronal connections in the developing nervous system relies on accurate navigation of growing axons. Since Ramón y Cajal's discovery of the growth cone, the phenomenon of axon guidance has been revealed as a coordinated operation of guidance molecules, receptors, secondary messengers, and responses driven by the dynamic cytoskeleton within the growth cone. With the advent of new and accelerating techniques, *Xenopus laevis* emerged as a robust model to investigate neuronal circuit formation during development. We present here the advantages of the *Xenopus* nervous system to our growing understanding of axon guidance.

Contents

1. Advantages of *Xenopus laevis* as a model organism for axon guidance

The complexity of neuronal networks has been a long-standing puzzle that has challenged scientists for centuries. Unveiling how this complex wiring is established in the mammalian brain has, in large part, relied on examination of simpler organisms with comparatively less intricate networks. For example, Ramón y Cajal's work on the chick brain produced the first description of the growth cone [1,2] and Harrison's work with frogs established the first neuronal culture system [3]. Furthermore, Sperry's pivotal experiment on frog retinal neuron regeneration [4] explained the chemospecificity of connections [5], which has been refined by further studies in systems such as *Xenopus* [6].

Xenopus, as a whole, offers an advantageous complementary vertebrate model, with a multitude of benefits. First of all, recently sequenced genomic data from *Xenopus* shows high similarity with the human genome [7]. There are several species of the *Xenopus* genus, but two have become increasingly popular in research. The diploid western-clawed *Xenopus tropicalis* offers advantages in genomic studies due to its smaller genome. On the other hand,

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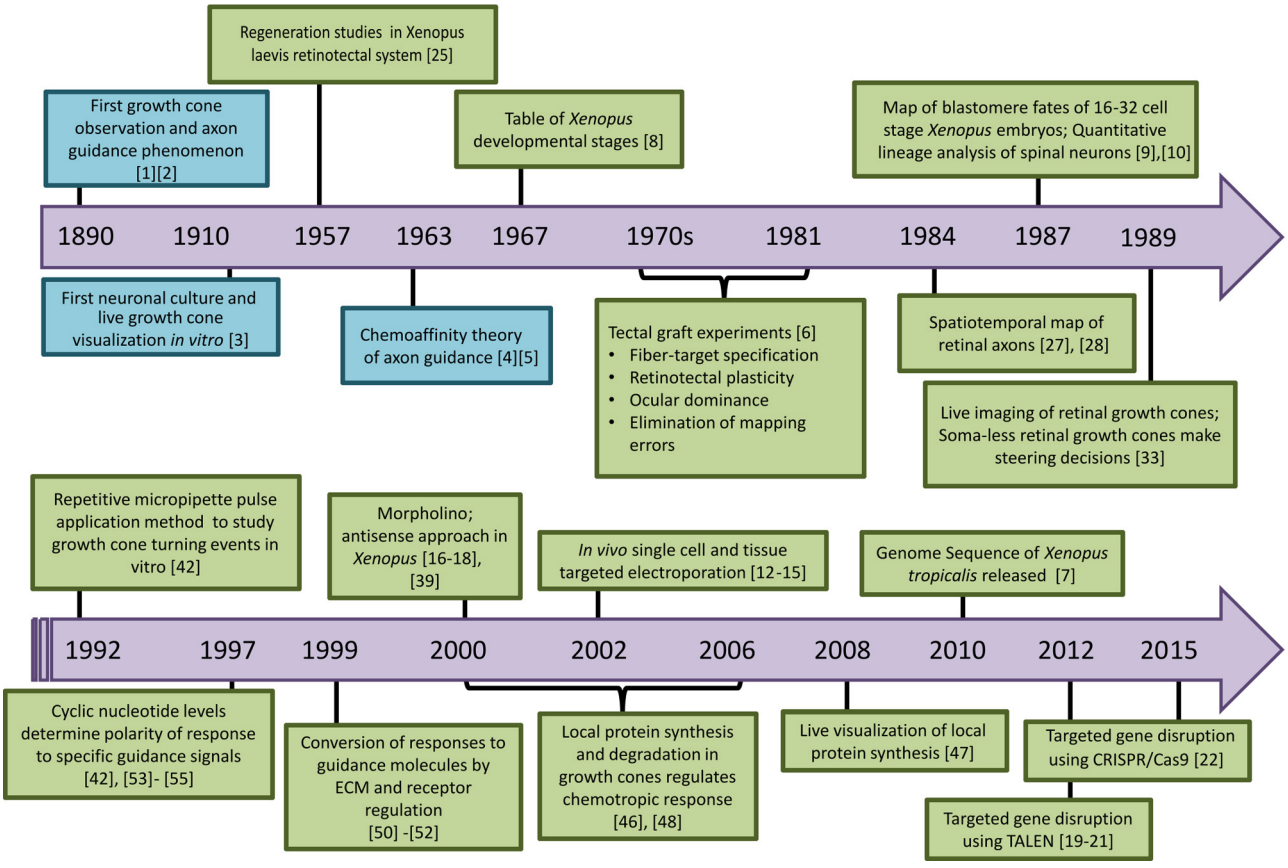

Fig. 1. Timeline of *Xenopus laevis* in axon guidance.
Key findings and important advances in *Xenopus laevis* (shown in green boxes) as a model organism during the development of the axon guidance field.

despite its large allotetraploid genome and longer maturation time, the African clawed frog *X. laevis* provides numerous advantages which make it a gold standard for studying axon guidance in development (Fig. 1).

The use of *X. laevis* in axon guidance research is advantageous for multiple reasons. Frog husbandry is relatively straightforward, and female frogs can be easily stimulated to produce eggs by simply injecting chorionic gonadotropin hormone. Eggs are comparatively large in diameter, 1–2 mm, and are produced in large quantities. Fertilization occurs *ex utero* and provides the opportunity to track and manipulate embryonic development at desired stages (Fig. 2A). Furthermore, embryos can tolerate extensive surgical manipulations varying from microinjection to cell and tissue transplantation. Based upon the given developmental stage and the known fate map of *X. laevis,* it is possible to target specific cell types. For instance, microinjecting mRNA at stages as early as the 1–4 cell stage results in global alteration of gene levels (Fig. 2B). Alternatively, injecting embryos at later stages, for example 16–64 cell, allows the restriction of gene manipulations to a more specific tissue [9,10] (Fig. 2C).

Compared to other systems, *X. laevis* neurons can be simply isolated and maintained at room temperature, permitting easy manipulation of live neurons as high resolution images are acquired, forgoing the need for strict incubation conditions such as those provided by CO_2 imaging chambers [11]. The primary benefit of *X. laevis* for these studies, however, is its large growth cones, which can be up to 10–30 μm in diameter and are perfect for clear and detailed analysis of subcellular cytoskeletal structures and dynamics. There may be no other vertebrate model system with growth cones as large and as easy to culture, manipulate, and image as *X. laevis*.

2. Manipulation of the *X. laevis* molecular arsenal

Delivery of molecules such as DNA, mRNA, antibodies, or fluorescent dextrans to modify expression of a particular gene or label a specific tissue is available via approaches such as microinjection or electroporation [12–15]. Genetic knockdown can be achieved via a variety of methods, the most common of which has been microinjection of antisense morpholino oligonucleotides (MOs). With the use of standard controls [16], MOs are advantageous tools to manipulate gene products [17], and they have been widely used for *Xenopus* gene knockdown since 2000 [18]. While the effects of MOs last for only a few days, however, the ability to achieve prolonged and heritable gene modifications is now possible with recently developed gene-editing nuclease systems. Transcription activator-like effector nucleases (TALENs), able to deliver high efficiency genetic knockout, have been used in *laevis* for multiple genes [19–21]. CRISPR-Cas9, for which it is much easier to produce guide RNA, and which displays even less off-target effects than TALENs [22], has been shown to be effective at disrupting pancreatic genes and pigment genes in *laevis* [23], and this technique will likely useful for investigations into neuronal genes as well. Together, as long as the proper control experiments are conducted, traditional MO approaches and/or newer CRISPR-Cas9 and TALEN systems of genetic manipulation provide complementary tools for efficient alteration of *X. laevis* proteins for axon guidance studies.

In addition to microinjection, electroporation allows manipulation of genes in later stage tissue and can be advantageous over other delivery methods. For instance, if the molecule of interest takes part in neuronal development as well as earlier stages of embryonic development, manipulation of its levels at blastomeric stages may result in lethality or embryonic abnormalities.