Neuroscience Letters 652 (2017) 82–93

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Review article

The African clawed frog *Xenopus laevis*: A model organism to study regeneration of the central nervous system

Dasfne Lee-Liu*, Emilio E. Méndez-Olivos, Rosana Muñoz, Juan Larraín*

Center for Aging and Regeneration, Millennium Nucleus in Regenerative Biology, Faculty of Biological Sciences, P. Universidad Católica de Chile, Alameda 340, Santiago, Chile

HIGHLIGHTS

- *Xenopus* brain and spinal cord regenerate in the larva, but not after metamorphosis.
- Optic nerve regeneration is maintained throughout frog lifespan.
- During metamorphosis, remodeling of brain stem supraspinal tracts takes place.
- Sox2$^+$ progenitor cells in the brain and spinal cord respond to injury.
- Studying *Xenopus* can provide important insights into improving neural regeneration.

ARTICLE INFO

Article history:
Received 26 July 2016
Received in revised form
18 September 2016
Accepted 28 September 2016
Available online 29 September 2016

Keywords:
Xenopus
Central nervous system regeneration
Spinal cord
Optic nerve
Brain
Regenerative model organisms

ABSTRACT

While an injury to the central nervous system (CNS) in humans and mammals is irreversible, amphibians and teleost fish have the capacity to fully regenerate after severe injury to the CNS. *Xenopus laevis* has a high potential to regenerate the brain and spinal cord during larval stages (47–54), and loses this capacity during metamorphosis. The optic nerve has the capacity to regenerate throughout the frog's lifespan. Here, we review CNS regeneration in frogs, with a focus in *X. laevis*, but also provide some information about *X. tropicalis* and other frogs. We start with an overview of the anatomy of the *Xenopus* CNS, including the main supraspinal tracts that emerge from the brain stem, which play a key role in motor control and are highly conserved across vertebrates. We follow with the advantages of using *Xenopus*, a classical laboratory model organism, with increasing availability of genetic tools like transgenesis and genome editing, and genomic sequences for both *X. laevis* and *X. tropicalis*. Most importantly, *Xenopus* provides the possibility to perform intra-species comparative experiments between regenerative and non-regenerative stages that allow the identification of which factors are permissive for neural regeneration, and/or which are inhibitory. We aim to provide sufficient evidence supporting how useful *Xenopus* can be to obtain insights into our understanding of CNS regeneration, which, complemented with studies in mammalian vertebrate model systems, can provide a collaborative road towards finding novel therapeutic approaches for injuries to the CNS.

Contents

1. Introduction 83
2. Advantages of *Xenopus* as a model organism to study central nervous system regeneration 83
   2.1. *Xenopus* as a laboratory model organism 83
   2.2. Development and remodeling of *Xenopus* central nervous system 84
3. Optic nerve regeneration throughout *Xenopus* lifespan: the role of extrinsic and intrinsic factors 84
4. Brain regeneration 87

* Corresponding authors at: Faculty of Biological Sciences, P. Universidad Católica de Chile, Alameda 340, Santiago, 8331150, Chile.
E-mail addresses: dnlee@uc.cl (D. Lee-Liu), jlarrain@bio.puc.cl (J. Larraín).

http://dx.doi.org/10.1016/j.neulet.2016.09.054
0304-3940/

5. Regeneration after spinal cord injury 88
5.1. Regenerative capacity during metamorphosis 88
5.2. Tissue and cellular origin of the regenerated spinal cord 89
5.3. Molecular mechanisms 90
6. Functional implications and conclusion 91
Conflict of interest 91
Acknowledgements 91
References 91

1. Introduction

An injury to the central nervous system (CNS) in humans has grim consequences: damage is mostly irreversible and accompanied by severe impairment of motor and sensory function [25,97]. It is therefore astounding that a group of organisms, namely amphibians and teleost fish, are capable of full recovery after severe injury to the CNS. Most importantly, it raises important questions: Which are the cellular and molecular mechanisms that allow such high regenerative potential? Could we harness this potential to improve regeneration in mammals, especially in humans?

Zebrafish and salamanders such as the newt and the axolotl are capable of CNS regeneration throughout their lifespans, while tailless frogs (order Anura) only have this potential during larval stages. Included in the latter are the African clawed frog *Xenopus laevis*, and the western or tropical clawed frog from the same genus, *Xenopus tropicalis* [6,19,52,67]. One of *Xenopus*' fundamental traits for the study of regeneration is that its regenerative potential is restricted to larval or tadpole stages (stages 47–54), and is lost during metamorphosis, when it turns into a froglet (developmental stages 56–66) [4,28,31,33,53,68]. One exception is the optic nerve, which has the potential to regenerate throughout *Xenopus*' lifespan [36,65]. The mechanisms that explain why *Xenopus* larvae are capable of CNS regeneration but froglets cannot are not yet fully understood, although the last two decades have had an increase in the use of *Xenopus* to study regeneration [19,52,67,86].

Here, we aim to review the work performed in *Xenopus*, mainly *X. laevis*, but also *X. tropicalis*, on spinal cord, brain and optic nerve regeneration, including a discussion on how the knowledge generated in these and other anurans can provide valuable information for the development of novel therapeutic approaches to treat CNS injuries in mammals. The field of spinal cord regeneration in particular has grown importantly in the past decades, and our knowledge of this process is increasing, for which the spinal cord will occupy a great part of this review. By presenting the knowledge gained from studying spinal cord, brain and optic nerve regeneration in *Xenopus*, we hope to provide convincing evidence on the contribution this model organism can be to advance our knowledge in the field. By understanding how regeneration competent organisms achieve CNS regeneration, we can obtain important insights into how neural regeneration and plasticity can be improved in mammals.

2. Advantages of *Xenopus* as a model organism to study central nervous system regeneration

2.1. Xenopus *as a laboratory model organism*

Before modern pregnancy tests, *X. laevis* frogs were used for this purpose. From the late 1940s to the 1970s, frogs were injected with the urine of possibly pregnant women, and the presence of human chorionic gonadotropin (hCG) induced the frogs to lay a large number of eggs within 4–12 h, indicating a positive result for the test [22,75]. Before frogs, immature female mice or rabbits were used. However, this test took longer and was more expensive, as ovary maturation indicated the positive result, for which animals needed to be sacrificed after each test [30]. The ease with which female frogs can be induced to lay eggs using commercially available hCG, which can then be fertilized *in vitro* for synchronized development, the relatively large size of the eggs and embryos (1.2 mm for *X. laevis*), and their *ex-utero* development has made *X. laevis* a classical model organism to study early vertebrate embryonic development [43]. Milestone scientific advances in cell and developmental biology have been performed in this organism. For example, in the late 1980s, a system for *in vitro* nuclear and chromatin assembly was developed in *X. laevis*, allowing the isolation of important components of the cell cycle, including the Meiosis maturation-promoting factor (MPF) [56,69]. During the 1990s, the first cloning of key signaling molecules that determine cell fate during dorsal-ventral patterning such as Chordin, Noggin and Follistatin, all antagonists of the Bone Morphogenetic Pathway (BMP) was also performed in *X. laevis* [18,43].

As a classical laboratory model organism, *Xenopus* poses the following experimental advantages: 1) Standard protocols for *Xenopus* laboratory breeding and husbandry have been available for several decades, which are comparably simpler and of a lower cost than those required by rodents [12]; 2) The availability of techniques to modify gene expression, like antisense morpholino oligonucleotides, the generation of transgenic animals [43,47], and recent developments on genome editing techniques like the CRISPR/Cas9 system [8,9,70]; 3) The availability of *Xenopus* genetic and genomic data to study genes, gene families and gene networks, including ESTs (expressed sequence tags), UniGene clusters and continually updated genomic sequences for both *X. laevis* and *X. tropicalis* [43,48]. The latter have allowed the use of high-throughput technologies in *Xenopus*, including RNA-Seq [3,15,53] and quantitative proteomics [74,90]. Furthermore, the National *Xenopus* Resource (NXR) and the European *Xenopus* Resource Centre (EXRC), among other stock centers, have an increasing resource of transgenic lines [92]. Importantly, amphibians including *Xenopus*, diverged more recently from amniotes (360 million years ago) than fish (over 400 million years ago), and frog and human genomes have extensive conserved synteny [43,44].

Specifically for the study of CNS regeneration, *Xenopus* has the following advantages: 1) The possibility to raise hundreds of regenerative larvae (3 weeks) and non-regenerative froglets (2 months), which can be used in comparative studies [11,33,68,72]; 2) The availability of reproducible CNS injury protocols, such as spinal cord transection, which dates back to 30 years ago [66], partial ablation of the brain [23,107,108], and optic nerve transection [36,55]; and the availability of an early fate map that allows targeting of specific reagents to a particular tissue. As a non-mammalian vertebrate, *Xenopus* lacks a somatosensory cortex and direct connections from the forebrain to the spinal cord, making its CNS circuitry simpler than that in mammals, but not as simple as invertebrate models, like *Drosophila melanogaster* or *Caenorhabditis elegans,* and still more amenable to elucidate cellular and molecular mechanisms.

It is also important to mention that *Xenopus* has some disadvantages, such as the long generation time for *X. laevis* (sexual maturation within 7–12 months), and the fact that it has an allote-