Development of genetic manipulation tools in Macrostomum lignano for dissection of molecular mechanisms of regeneration

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CHAPTER 1

General introduction and the outline of the thesis
OPENING REMARKS

Influencing the genomes of various organisms has been present in the human history for thousands of years. Current standing hypothesis considers the domestication of the gray wolf dating back to around 12000 BC as the first event when humans had influenced the process of genetical change via artificial selection [1]. With the development of our civilization humans were able to affect the surrounding biology in more various and sophisticated ways including selective breeding, hybridization and grafting [2]. The breakthrough came with the onset of modern genetics. The discovery of restriction enzymes in 1970 [3] started the genetic engineering as we know this term today. In other words, people finally started to understand what they were doing in terms of genetic manipulation. Already in 1973, the first genetically modified bacteria were created [4] and only a year later the first transgenic mouse was born [5]. With almost every major discovery in the field of molecular biology, transgenic techniques were improving. The use of, already known, transposable elements, discovery of polymerase chain reaction (PCR), fluorescent proteins, Transcription Activator-Like Effector Nucleases (TALENs) or the most recent breakthrough – Clustered Interspaced Palindromic Repeats/Cas9 (CRISPR/Cas9), all pushed the boundaries of what scientists could decipher about the biology of different organisms [6–11]. Throughout the years genetic manipulation rose to one of the hallmarks of the scientific progress. Present in the popular culture, movies, comic books, sci-fi novels, modifying genomes represents one of humanities great achievements. The reality, however, tends to be a lot different than what is presented in the cinemas. Today, after almost fifty years, despite the magnificent progress that was achieved in the field of transgenics, we still have plenty of blank spots that can be filled in, some of which requiring a proper approach rather than a scientific breakthrough.

One of such blank spots is the genetic manipulation in flatworms. Flatworms have been known for their regenerative capabilities for over a century [12], and they are a popular choice when it comes to model organisms. Their unique ability comes from a population of adult stem cells referred to as neoblasts [13, 14]. One can imagine that in-depth characterization of neoblasts should improve our understanding of processes such as regeneration and stem cell differentiation. There has been a lot of effort put into deciphering the functioning of these cells, proving their pluripotency [15] or showing that the population is, in fact, heterogeneous [16]. Despite the progress we are still unable to reliably isolate or tag the neoblasts. One of the reasons for this is the lack of available transgenic techniques necessary to efficiently modify the flatworm genome. This also severely hampers our understanding of the biology of flatworms, currently making them a less attractive model for any genetic studies.

This thesis is focused on *Macrostomum lignano*, a marine free-living flatworm with high regenerative potential. The overall aim of this work was to improve on the currently available techniques for flatworm genetic studies by developing a molecular toolkit for genetic manipulation and provide sufficient evidence to make *M. lignano* is a versatile and robust model organism to study regeneration, stem cells, ageing and many other fields of biology.
THESIS OUTLINE
The thesis is structured as follows:

Chapter 2 introduces *Macrostomum lignano* as a model organism giving an overview on the biology of the animal. It also explores the potential research areas that could benefit from using the flatworm as either substitute or supplementary model organism to answer the questions related to regeneration and stem cell biology.

Chapter 3 presents the development and implementation of the transgenic techniques for *Macrostomum lignano*. It introduces the molecular toolkit for genetic manipulation in *M. lignano*. The toolkit is focused on the microinjection approach, which is shown to be robust, reproducible and reliable. Up to today it is the only available method to create stable transgenic flatworms. This chapter also presents the most up-to-date *M. lignano* genome assembly together with the updated transcriptome assembly and genome annotation. The molecular toolkit from this chapter will be further expanded and tested in throughout the thesis.

Chapter 4 explores the use of the *piggyBac* transposon system to facilitate stable integration of the desired DNA fragments. It focuses on advantages that this approach offers as compared to the method presented in Chapter 3. It also highlights new directions in which further development of the genetic manipulation techniques for *M. lignano* can go.

Chapter 5 demonstrates the influence of the temperature on the basic aspects of the flatworm biology, including development, reproduction and regeneration. It also presents how transgenic animals, made using the techniques described in chapter 3 are used for research purposes. Finally, it shows how temperature manipulation can facilitate future experiments that will be performed using *Macrostomum lignano*.

Chapter 6 is focused on how transgenic lines expressing GFP under tissue-specific promoters can be used to isolate desired cell populations by FACS and characterize them using RNA-seq. The specificity of the technique enables more detailed characterization of flatworm neoblasts by more precisely separating them from the germline. This chapter presents practical application of the available transgenic lines. It also highlights how data obtained using *M. lignano* can supplement current findings and point the way for future research.

Chapter 7 summarizes the findings presented in this thesis and gives a brief overview of the obtained results. It also explores their relevance and potential impact on future research.
REFERENCES


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