ELSEVIER

Seminars in Cell & Developmental Biology 18 (2007) 543–558

seminars in
CELL & DEVELOPMENTAL
BIOLOGY

www.elsevier.com/locate/semcdb

Invited article

Molecular genetics of pituitary development in zebrafish

Hans-Martin Pogoda[a], Matthias Hammerschmidt[a,b,*]

[a] *Max-Planck Institute of Immunobiology, Stübeweg 51, D-79108 Freiburg, Germany*
[b] *Institute for Developmental Biology, Cologne University, Gyrhofstrasse 17, D-50923 Cologne, Germany*

Available online 19 April 2007

Abstract

The pituitary gland of vertebrates consists of two major parts, the neurohypophysis (NH) and the adenohypophysis (AH). As a central part of the hypothalamo-hypophyseal system (HHS), it constitutes a functional link between the nervous and the endocrine system to regulate basic body functions, such as growth, metabolism and reproduction. The development of the AH has been intensively studied in mouse, serving as a model for organogenesis and differential cell specification. However, given that the AH is a relatively recent evolutionary advance of the chordate phylum, it is also interesting to understand its development in lower chordate systems. In recent years, the zebrafish has emerged as a powerful lower vertebrate system for developmental studies, being amenable for large-scale genetic approaches, embryological manipulations, and in vivo imaging.

Here, we present an overview of current knowledge of the mechanisms and genetic control of pituitary formation during zebrafish development. First, we describe the components of the zebrafish HHS, and the different pituitary cell types and hormones, followed by a description of the different steps of normal pituitary development. The central part of the review deals with the genes found to be essential for zebrafish AH development, accompanied by a description of the corresponding mutant phenotypes. Finally, we discuss future directions, with particular focus on evolutionary aspects, and some novel functional aspects with growing medical and social relevance.

Keywords: Zebrafish; Pituitary; Adenohypophysis; Neurohypophysis; Lineage specification; Pitx3; Shh; Fgf3; Ascl1a; Pit1; Eya1

Contents

* Corresponding author. Tel.: +49 761 5108 495; fax: +49 761 5108 744.
E-mail address: hammerschmid@immunbio.mpg.de (M. Hammerschmidt).

1084-9521/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.semcdb.2007.04.004

1. The zebrafish as a powerful system for genetics, functional genomics, and in vivo imaging

Why study pituitary development in the tropical fresh water fish and aquarium pet *Danio rerio*, commonly called zebrafish, while a lot is already known about the molecular and genetic control of pituitary formation in the prime mammalian model system, the mouse [1,2]?

First, there is an evolutionary aspect. Although a recent report describes a potential functional equivalent in the fruit-fly Drosophila melanogaster [3], it is commonly believed that the pituitary gland as a central component of the hypothalamo-hypohyseal system (HHS) is an evolutionary rather recent advance of the chordate phylum. In this light, to better understand the evolution of this important gland, it is reasonable to compare its ontogeny in non-vertebrate chordates, as well as in lower and higher vertebrates (see also Section 5.1).

Second, compared to the mouse system, the zebrafish offers certain complementary advantages that might prove as useful to unravel thus far unidentified mechanisms and regulators of pituitary development. Thus far, approximately 20 genes have been found to be directly required for the development of the pituitary gland in mouse [1,2]. Most of them have been identified via recombinant gene targeting or transgenic approaches ("reverse genetics"), while no saturating systematic genetic analysis has been carried out as yet, due to the extreme costs and space demands of such an enterprise. However, large-scale "forward genetic" screens can be rather easily performed in zebrafish. Compared to mouse, zebrafish can be kept at much higher density. In addition, fecundity is much higher, with single females giving weekly clutches of 100–500 synchronously developing eggs, which is more than enough to identify mutations that segregate in Mendelian ratios. Although different protocols have been established, the standard forward genetics method is a three-generation (F3) screen after mutagenesis with *N*-ethyl-*N*-nitrosourea (ENU), which induces single nucleotide exchanges at random positions of the genome [4]. Mutated genes have to be subsequently identified via meiotic mapping and positional cloning [5], which can still be a laborious task. However, it has been enormously facilitated with the near completion of the genome and the genome-wide establishment of BAC contigs and genetic and physical maps. A reasonable alternative to ENU is insertional mutagenesis, which has the advantage that mutated genes can be more easily cloned, as they are tagged by the retroviral insert [6,7].

In addition, different "reverse genetics" approaches have been established, such as TILLING (targeted induced local lesions in genomes), where a library of ENU-mutagenized F1 genomes is screened for mutations in particular genes via PCR-amplification and sequencing [8]. Alternatively, specific gene products can be inactivated via injection of chemically modified antisense morpholino oligonucleotides (MOs) [9]. In adaptation to "mutants", such MO-injected embryos are called "morphants". Compared to mutants, they can be generated in a much shorter time, and screens systematically knocking down expressed zebrafish genes are ongoing [10]. However, for the pituitary, this approach is restricted to early stages of development, as MOs are only effective during the first 3 days after injection at the one-cell stage.

Another advantage of the zebrafish is its rapid and external development, and the optical transparency of its embryos and larvae, which makes it well amenable for in vivo imaging. For the pituitary, this approach has become particularly useful after the generation of transgenic reporter lines in which particular AH cell types are fluorescently labeled by GFP or RFP under the control of hormone gene promoters [11,12] (Fig. 2A and B). These tools do not only allow in vivo observation of pituitary morphogenesis, but also quantitative measurements of the effects of mutations or chemical treatments on pituitary cell proliferation or hormone gene activation.

2. Composition and function of the zebrafish pituitary

2.1. The hypothalamo-hypophyseal system

Similar to its position in mammals, the pituitary gland or hypophysis of adult zebrafish is located in a bony hollow beneath the hypothalamus, just posterior to the optic chiasm (Fig. 1E and F). At larval stages, it is positioned directly above the fenestra of the neurocranial cartilage, separated from the underlying oral cavity epithelium by a layer of collagen fibrils (Fig. 1C) [13]. As a central part of the HHS, the pituitary links the nervous and the endocrine systems to regulate an array of vital processes including body homeostasis, growth, and reproduction. As in mammals, the HHS of teleosts is separable into three major divisions: the hypothalamus, which is part of the diencephalon, the neurohypophysis (NH), which derives from the ventral diencephalon, representing the neural compartment of the pituitary, and the adenohypophysis (AH), which is the non-neural part of