Available online at www.sciencedirect.com
ScienceDirect
General and Comparative Endocrinology 155 (2008) 176–189
GENERAL AND COMPARATIVE ENDOCRINOLOGY
www.elsevier.com/locate/ygcen

An F-domain introduced by alternative splicing regulates activity of the zebrafish thyroid hormone receptor α ☆

Sachiko Takayama [a], Ute Hostick [a,b], Melissa Haendel [b,1], Judith Eisen [b], Beatrice Darimont [a,*]

[a] *Institute of Molecular Biology, University of Oregon, Eugene, OR 97403-1229, USA*
[b] *Institute of Neuroscience, University of Oregon, Eugene, OR 97403-1229, USA*

Received 20 October 2006; revised 19 March 2007; accepted 13 April 2007
Available online 27 April 2007

Abstract

Thyroid hormones (THs) play an important role in vertebrate development; however, the underlying mechanisms of their actions are still poorly understood. Zebrafish (*Danio rerio*) is an emerging vertebrate model system to study the roles of THs during development. In general, the response to THs relies on closely related proteins and mechanisms across vertebrate species, however some species-specific differences exist. In contrast to mammals, zebrafish has two TRα genes (*thraa*, *thrab*). Moreover, the zebrafish *thraa* gene expresses a TRα isoform (TRαA1) that differs from other TRs by containing additional C-terminal amino acids. C-terminal extensions, called ''F domains'', are common in other members of the nuclear receptor superfamily and modulate the response of these receptors to hormones. Here we demonstrate that the F-domain constrains the transcriptional activity of zebrafish TRα by altering the selectivity of this receptor for certain coactivator binding motifs. We found that the F-domain of zebrafish TRαA1 is encoded on a separate exon whose inclusion is regulated by alternative splicing, indicating a regulatory role of the F-domain *in vivo*. Quantitative expression analyses revealed that TRαA1 is primarily expressed in reproductive organs whereas TRαB and the TRαA isoform that lacks the F-domain (TRαA1-2) appear to be ubiquitous. The relative expression levels of these TRα transcripts differ in a tissue-specific manner suggesting that zebrafish uses both alternative splicing and differential expression of TRα genes to diversify the cellular response to THs.

Keywords: Thyroid hormone; Thyroid hormone receptor (TR); Isoforms; F-domain; Zebrafish (*Danio rerio*); Alternative splicing; Tissue-specific expression; Coactivator interaction

1. Introduction

Thyroid hormones (THs) are essential for normal development, differentiation, and metabolic balance of vertebrates (Yen, 2001). In amphibians and some fish THs are the key regulators of metamorphosis, whereas in mammals hypothyroidism leads to cretinism, mental retardation, and deafness (Pitt-Rivers and Tata, 1959; DeLong, 1996; Power et al., 2001). Although the general mechanisms of TH physiological actions are known, the roles of these hormones during development remain largely elusive (Chan and Kilby, 2000; Tata, 1999). Recently, zebrafish has been introduced as a novel non-mammalian model system to facilitate the manipulation, dissection and genetic analysis of TH activities during development (Brown, 1997; Essner et al., 1997, 1999; Liu et al., 2000; Liu and Chan, 2002; Lam et al., 2005). Manipulation of TH levels provided evidence that THs are of particular importance during the embryonic-larval and larval-juvenile transitions (Brown,

☆ This work was supported by grants from the Medical Research Foundation of Oregon to B.D. and National Institute of Health to J.E. (HD22786). M.H. was funded by a postdoctoral fellowship from the National Institute of Health (NS11170).
* Corresponding author. Fax: +1 541 346 5891.
E-mail address: bead@molbio.uoregon.edu (B. Darimont).
[1] Present address: Zebrafish Information Network, University of Oregon, Eugene, OR 97405-5291, USA.

0016-6480/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2007.04.012

1997; Liu and Chan, 2002). Chemical disruption of TH production in zebrafish embryos and larvae results in stunted growth, retarded head cartilage development, paired fin elongation, curled tails, changes in the distribution and structure of melanophores, and impairs the development of the thymus (Brown, 1997; Liu and Chan, 2002; Lam et al., 2005; Elsalini and Rohr, 2003). Zebrafish embryos that have been treated with exogenous THs do not develop swimbladders, have smaller and bent bodies, severely retarded gastrointestinal system development and fewer and smaller melanophores (Liu and Chan, 2002). Complete absence or high concentrations of TH during zebrafish embryogenesis is lethal (Liu and Chan, 2002).

The cellular activity of THs is exerted by two thyroid hormone receptors, TRα and TRβ (Lazar, 1993; Marchand et al., 2001). Differential promoter usage and alternative splicing enables the TRα and β genes to give rise to multiple isoforms (Flamant and Samarut, 2003). The temporal and regional expression of these isoforms constitutes an important mechanism for the stage- and tissue-specific regulation of cellular responses to THs (Liu et al., 2000; Yamano and Miwa, 1998; Chassande et al., 1997; Buchholz et al., 2006). TRs are hormone regulated transcription factors that belong to the nuclear receptor superfamily (Aranda and Pascual, 2001). Like all nuclear receptors, TRs have a modular structure and contain highly conserved DNA (DBD) and ligand binding (LBD) domains as well as a less conserved N-terminal domain (Zhang and Lazar, 2000). Depending on the type of thyroid hormone response element (TRE), TRs can bind DNA as monomers, homodimers, or heterodimers with retinoid X receptors (RXRs) (Lazar et al., 1991; Bugge et al., 1992; Rastinejad et al., 1995).

THs control the activities of TRs by inducing conformational changes that regulate the interaction of the TR LBD with corepressors and coactivators (Renaud and Moras, 2000; Privalsky, 2004). In the absence of hormone, TRs interact with corepressors that are released upon hormone binding and replaced by coactivators (Glass and Rosenfeld, 2000). The hormone-dependent transition of TRs from transcriptional repressors to transcriptional activators plays a decisive role during development (Mai et al., 2004). The dual activity rationalizes why TRs are usually expressed prior to the onset of fetal TH production. Moreover, this mechanism also explains that the absence of TRs, which prevents the active repression and activation of TR target genes, is physiologically less detrimental than reduced levels of THs, which result in constitutive repression of TR target genes.

The general mechanisms of TH actions appear to be similar across vertebrates; however, some species-specific variations have been identified. One of these differences concerns the onset of zygotic TH production and the availability of THs before this point. Although there is evidence that mammalian embryos are exposed to maternal THs and that in transgenic mice TH-dependent reporters can be activated before the onset of zygotic TH production, TH levels are considered to be low during early mammalian development (Chassande et al., 1997; de Escobar et al., 2004). In contrast, oocytes from fish and other non-mammalian vertebrates can contain large amounts of maternal THs (Power et al., 2001). It is still unclear whether these maternal THs are available to activate TRs during embryogenesis. Another species-specific difference is the number and expression of the TR genes and details in the structure of TRs. Due to ancestral gene duplication events, some non-mammalian vertebrate species such as the African clawed frog (*Xenopus laevis*) and the Japanese flounder (*Paralichthys olivaceus*) have several TRα-encoding genes (Yaoita and Brown, 1990; Yamano et al., 1994; Yamano and Inui, 1995). Similarly, the genome sequencing project (http://www.sanger.ac.uk) recently identified an additional TRα-encoding gene in zebrafish (GenBank Accession No. NW_001510851). Based on available cDNAs, both zebrafish TRα genes appear to be expressed and give rise to at least two TRα products (TRαA1; TRαB).[2]

A seemingly unique feature of zebrafish TRαA1 is the extension of α-helix H12 at the TRα C-terminus by 17 amino acids (Essner et al., 1997; Marchand et al., 2001). C-terminal extensions, called ''F domains'', are common in other members of the nuclear receptor superfamily. With the exception of some steroid hormone receptors, for most nuclear receptors the sequence and size of F-domains are highly variable and can differ substantially even for closely related receptors. Thus far, the structures of only a few steroid hormone receptor F-domains have been solved (Williams and Sigler, 1998; Bledsoe et al., 2002; Kauppi et al., 2003) (Fig. 1a). Consistent with these structures, mutational studies indicate that F-domains regulate the response of steroid hormone receptors to ligands by modulating the interactions with coactivators and corepressors (Montano et al., 1995; Schwartz et al., 2002; Peters and Khan, 1999). Mutational analyses suggested that F-domains of other nuclear receptors have similar functions (Suaud et al., 1999; Sladek et al., 1999; Ruse et al., 2002; Farboud and Privalsky, 2004).

In this study we investigated whether the F-domain of zebrafish TRαA1 plays a role in regulating the activity of zebrafish TRα and contributes to the stage- and tissue-specific regulation of cellular responses to THs.

[2] Due to the identification of the *thrab* gene (GenBank Accession No. NW_001510851), the original zebrafish TRα gene (GenBank Accession No. NP_571471) is now called *thraa*. As shown in this study, *thraa* gives rise to at least two products: the F-domain encoding *thraa1* (TRαA1, previously called TRα1 (Essner et al., 1997); GenBank Accession No. U54796) and *thraa1-2* (TRαA1-2; GenBank Accession No. DQ991961). Evidence for the expression of *thrab* is given by GenBank Accession Nos. DQ017632 and DQ991962 (this study).