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Zebrafish *ftz-f1a* (*nuclear receptor 5a2*) functions in skeletal muscle organization

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Abstract

Fushi-tarazu factor 1a (Ftz-F1a, Ff1a, Nr5a2) is a nuclear receptor with diverse functions in many tissues. Here, we report the function of *ff1a* in zebrafish muscle differentiation. In situ hybridization revealed that *ff1a* mRNA was present in the adaxial and migrating slow muscle precursors and was down-regulated when slow muscle cells matured. This expression was under the control of *hedgehog* genes, expanded when *hedgehog* was increased and missing in mutants defective in genes in the Hedgehog pathway like *you-too* (*yot*), *sonic you* (*syu*), and *u-boot* (*ubo*). Blocking *ff1a* activity by injecting a deleted form of *ff1a* or an antisense *ff1a* morpholino oligo into fish embryos caused thinner and disorganized fibers of both slow and fast properties. Transient expression of *ff1a* in *syu*, *ubo*, and *yot* embryos led to more fibril bundles, even when slow myoblasts were transfated into fast properties. We showed that *ff1a* and *prox1* complemented each other in slow myofibril assembly, but they did not affect the expression of each other. These results demonstrate that *ff1a* functions in both slow and fast muscle morphogenesis in response to Hedgehog signaling, and this function parallels the activity of another slow muscle gene, *prox1*.

Keywords: NR5A2; *ff1a*; LRH-1; FTF; Hedgehog; *ubo*; *syu*; *yot*; *prox1*; Twitch fiber

Introduction

Vertebrate skeletal muscles are broadly categorized into two main groups of cells, fast (white) and slow (red), based on their structural and functional properties. Slow contracting, oxidative muscle cells are characterized by unique structural proteins; slow isotype myosin heavy chains are involved in slow and sustained movements, whereas fast muscle cells are characterized by their fast isotype myosin heavy chains and by rapid response to stimulus (Francis-West et al., 2003).

Amniote slow and fast muscles develop together from a single origin, therefore it is difficult to delineate their separate mechanisms of development (Hughes and Salinas, 1999). In zebrafish, fast and slow muscle cells originate from unique precursor cells, namely lateral mesoderm cells and adaxial cells, respectively, and occupy distinct portions of the somites (Devoto et al., 1996; Du et al., 1997). After segmentation of each somite, adaxial cells extend dorso-ventrally spanning the entire width of the somite. Most of these cells later migrate radially away from the notochord to the superficial layer of the somite to form a monolayer of embryonic slow muscle cells (non-muscle pioneer cells). Two to six adaxial cells, termed as muscle pioneer cells, stay in close proximity to the notochord. Subsequently, fast muscle cells start to differentiate. Such distinct differences between these two cell types make zebrafish a good model for studying myogenesis.

The development of slow muscle cells is governed by a cascade of molecular events. In vertebrates, the Hedgehog (Hh) family of secretary glycoproteins derived from axial structures (notochord and floor plate) is required for adaxial cells to adopt early slow muscle fate (Lewis et al., 1999). Although much is known about the early specification of

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slow muscle cells, very few studies have been done on the molecular mechanisms involved in later differentiation of muscle fiber and myofibril formation, except one report that describes the function of a homeobox gene *prox1* in the regulation of slow myofibril assembly in zebrafish (Roy et al., 2001). Signals that regulate fast muscle development in zebrafish have not been fully defined, except the knowledge that slow muscle migration may affect fast muscle morphogenesis, and when Hh is blocked fast muscle cells do not elongate (Henry and Amacher, 2004).

In zebrafish, mutants defective in Hh signaling are also called u-type mutants because of their characteristic u-shaped rather than the normal v-shaped somites due to the loss of myoseptum (van Eeden et al., 1996). The *sonic you* (*syu*) mutant defective in the *shh* gene has only a few slow muscle cells (Schauerte et al., 1998). The *you-too* (*yot*) mutation for the *gli2* gene, which encodes a transcription factor mediating Hh signaling involved in slow muscle induction, results in total ablation of slow muscle precursor cells (Du and Dienhart, 2001; Lewis et al., 1999). *U-boot* (*ubo*) is another gene important for slow fiber specification (Roy et al., 2001). Because of the slow muscle defects, these u-type mutants serve as a good tool to study skeletal muscle development.

Fushi-tarazu factor 1 (Ftz-f1) is a zinc finger protein in the orphan nuclear receptor family. It was first identified as a regulator of the *fushi-tarazu* segmentation gene during early embryogenesis in *Drosophila* (Ueda et al., 1990). Members of this gene family are classified as Nuclear Receptor 5A (NR5A) (Nuclear-Receptors-Nomenclature-Committee, 1999). We have recently characterized zebrafish *ftz-f1a* (*ff1a*), which belongs to the *nr5a2* family (Kuo et al., 2005). The mammalian ortholog of Ftz-f1a is LRH-1 in mouse or FTF in rat. This protein is a transcription factor involved in the activation of genes in bile acid and lipid metabolism (Fayard et al., 2004), hepatitis virus transcription and replication (Cai et al., 2003), pancreatic development (Annicotte et al., 2003), and endocrine functions (Hinshelwood et al., 2003). We have previously shown that *ff1a* is expressed in liver, pancreas, intestine, spinal neurons, mandibular arches, and hypothalamus in zebrafish (Lin et al., 2000). In the present study, we demonstrate that *ff1a* is expressed in zebrafish slow muscle cells in a dynamic pattern in response to Hh signaling and that it plays an important role during skeletal muscle development. This study adds a new dimension to the known diverse functions of *nr5a2*.

Materials and methods

Wild and mutant zebrafish strains

AB (Wild-type) and smu^{b577} fish strains were obtained from Oregon Fish Facility; TL (wild type), ubo^{tp39c}, and yot^{ty119} mutants were obtained from Tuebingen, Germany; syu^{tq252} from Singapore. Fish were bred and maintained at 28.5°C on a 14–10 h light/dark cycle.

Plasmids

Zebrafish *ff1a* can be alternatively spliced as four isoforms *IA*, *IB*, *IIA*, and *IIB*, but only form *IIA* was detected in embryonic stages (Lin et al., 2000). Previous studies have shown that the full-length *IIA* form of *ff1a* activates transcription, whereas a truncated *IIB* form of zebrafish *ff1a*, which lacks the transactivation domain, inhibits full-length *ff1a* activity (Liu et al., 1997). This truncated zebrafish *ff1a* (*dff1a*) serves as a dominant negative form blocking the function of all the isoforms of *ff1a* (Liu et al., 1997). The full-length *ff1a IIA* (*ff1a*), *dff1a*, and *β-galactosidase* were each cloned into a *pCS2* vector for microinjection, and *pCS2prox1* was a gift from Dr. Woon-Khiong Chan, Singapore. Plasmids containing *mShh* and *PKI* in *pSP4t* vector (Hammerschmidt et al., 1996) have been described previously.

Forskolin treatment

Wild-type embryos were treated with 0.9 mM forskolin dissolved in 1.5% dimethylsulfoxide in embryo medium from 4–5 h post fertilization (hpf) to 18–20 hpf. Control embryos were treated with 1.5% dimethylsulfoxide in embryo medium for the corresponding period of time.

Microinjection and in situ hybridization

Capped RNA for microinjection was synthesized by in vitro transcription according to manufacturer's protocol (mMESSAGE mMACHINE T7 or SP6, Ambion Inc., Austin, Texas, USA). Antisense *ff1a* morpholino oligonucleotide with the sequence of CTGACTCGACTTTAGGCAGCATGAC was used to block translation of *ff1a IIA* and *IIB* (Lin et al., 2000). Fertilized eggs at one- to four-cell stages were microinjected with 50–200 pg RNA or 12–23 ng morpholino oligo per embryo. Embryos were fixed at the desired stages in 4% paraformaldehyde overnight at 4°C and washed with phosphate-buffered saline and stored in methanol at −20°C until use. Sense and antisense probes were synthesized using in vitro transcription from linearized plasmids using RNA polymerase and digoxigenin RNA labeling mix according to manufacturer's protocol (Roche Diagnostic's kit, Germany). Whole mount in situ hybridization was carried out as previously described (Chiang et al., 2001).

Immunostaining

Immunostaining was performed following the established method (Barresi et al., 2000; Devoto et al., 1996). F59 IgG, which recognizes preferentially slow and weakly fast muscle fibers (Miller et al., 1989), and S58 IgA, which