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DIFFERENTIAL EXPRESSION OF SYNAPSIN GENES DURING EARLY ZEBRAFISH DEVELOPMENT

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Abstract—Synapsins are a family of synaptic vesicle (SV)-associated phosphoproteins that have been identified in several vertebrates and invertebrates. We report here the cloning and expression of synapsin family genes in the zebrafish *Danio rerio*. We identified the complete coding sequence of synapsin 3, which is not present in the currently available genome, and characterized and annotated the synapsin gene family in the zebrafish *D. rerio*. By means of whole-mount *in situ* hybridization, we showed the spatio-temporal expression of synapsin genes at three different time points during early embryonic development: 20–24 h postfertilization (hpf), 30–33 hpf, and 3 days postfertilization (dpf). As very few data are available describing the expression of synapsin family genes during CNS development in vertebrate models, our results may help to achieve a better understanding of the complex functions of these molecules. Finally, new interesting evidence from our temporal gene expression studies suggests that synapsins have also maternal functions.

Key words: *in situ* hybridization, synapsin, nervous system, *Danio rerio*, Syn locus evolution.

INTRODUCTION

Synapsins are phosphoproteins that are associated with synaptic vesicles (SVs) and conserved in vertebrate and invertebrate species (De Camilli et al., 1990; Kao et al., 1999; Candiani et al., 2010). Synapsins are involved in neurotransmitter release, synaptic plasticity, neurite elongation and synapse formation (Ferreira et al., 1994). Vertebrate synapsins are encoded by three different genes, *Syn1*, *Syn2* and *Syn3*, while invertebrates have a single synapsin gene (Candiani et al., 2010). At least 10 distinct alternative transcripts (*Syn1a/b*, *Syn2a/b*, *Syn3a–f*) (Kao et al., 1999) have been identified in vertebrates. Synapsins are present in the majority of synapses in the central and peripheral nervous systems (De Camilli et al., 1990; Ferreira et al., 1994). In mammals, the three synapsin genes show a distinct temporal expression pattern in neurons. *Syn1* and *2* are upregulated at the onset of synaptogenesis and remain elevated in mature neurons (Kao et al., 1998; Porton et al., 1999). *Syn3* is expressed early during neuronal development, and its expression is downregulated in mature neurons. In addition, synapsin 3 seems to regulate axonal growth and growth cone size in developing neurons (Ferreira et al., 2000; Feng et al., 2002) and it is also distributed in the soma of neurogenic regions of the hippocampus and the rostral migratory stream (Kao et al., 2008). Such data are indicative of an early role for Syn3 in neural progenitor cell development of the adult hippocampus. *Syn1* and 2 have partially overlapping functions, and defects in both are associated with epileptic and autistic-like phenotypes in mice (Fassio et al., 2011; Cambiaghi et al., 2013; Corradi et al., 2014). Several studies have also documented the association of Syn3 defects with multiple sclerosis and neuropsychiatric diseases (Otaegui et al., 2009; Chen et al., 2009). Outside of the nervous system, secretory-pancreatic β cells (Matsumoto et al., 1999) and chromaffin cells (Haycock et al., 1988) contain synapsins.

The three mammalian synapsin genes are each associated with a TIMP gene. Such genomic organization is also conserved for the invertebrate synapsin gene. However vertebrates contain genes encoding synapsins and TIMPs, some of which are nested and others are independent. This gene arrangement indicates that the ancestral Syn–Timp locus has undergone duplications during vertebrate evolution (Yu et al., 2003). The mammalian genome has undergone the two rounds of whole-genome duplication that occurred in the common ancestor of vertebrates, whereas a third genome duplication occurred in the stem

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Abbreviations: ISH, *in situ* hybridization; PCR, polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; SV, synaptic vesicle.

Abbreviations used in figures: allg: anterior lateral line ganglion; cep, cerebellar plate; cg, cranial ganglia; d, diencephalon; dt, dorsal thalamus; e, epiphysis; emt, eminetia thalami; gc, retinal ganglion cell layer; h, hypothalamus; ha, habenula; hc, caudal hypothalamus; inl, inner nuclear layer; irf, inferior reticular formation; mo, medulla oblongata; n, region of nucleus of medial longitudinal fascicle; ob, olfactory bulb; oe, olfactory epithelium; og, otic ganglion; ov, otic vesicle; p, pallium; pllg, posterior lateral line ganglion; po, preoptic region; pr, pretectum; pt, posterior tuberculum; ptv, ventral part of posterior tuberculum; rh, rhombomeres; rl, rhombic lip; rve, rhombencephalic ventricle; s, subpallium; sc, spinal cord; t, telencephalon; teo, tectum optic; teg, midbrain tegmentum; tg, trigeminal ganglion; ts, torus semicircularis; tve, telencephalic ventricle; vt, ventral thalamus.

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lineage of teleost fishes (Postlethwait et al., 2000; Meyer and Van de Peer, 2005). The teleost *Takifugu rubripes*, has four synapsin genes: *Syn1*, *Syn2a*, *Syn2b*, and *Syn3* (Yu et al., 2003). A comparison of the Fugu and human Syn loci indicates that the Fugu contains an additional Syn gene (*Syn2b*). While the two Syn2 proteins, Syn2a and Syn2b, are generated by alternative splicing of the mammalian gene, they are encoded separately by the duplicate Fugu and zebrafish *Syn2a/b* paralog genes.

With regard to the zebrafish synapsin genes, only *syn1*, *syn2a* and *syn2b* are known, and their expression has been analyzed only in the spinal cord of embryos at 17–25 hpf (Courtney Easley-Neal et al., 2013). However, a comprehensive study on the developmental timing of synapsin gene expression in animal models and in particular in anamniotes does not exist. Furthermore, a large body of evidence comes from cell cultures and not from *in vivo* models. Thus, to illuminate the role of synapsins in the nervous system and to contribute to the clarification of the cellular dysfunctions underlying several cognitive diseases, we studied synapsin gene expression during the development of the zebrafish *Danio rerio*.

Here, for the first time, we report the mammalian ortholog of *syn3* in the zebrafish (*D. rerio*) genome. We cloned all four zebrafish synapsin genes (*syn1*, *syn2a*, *syn2b*, and *syn3*) and examined the temporal and spatial expression at different developmental stages. We also analyzed the correlation of synapsin and TIMP genes in the zebrafish genome to understand the evolution of these genes during vertebrate genome duplications.

EXPERIMENTAL PROCEDURES

Animal collection and RNA preparation

Embryos of the AB strain were grown at 28.5 °C under standard procedures (Westerfield, 2000) and staged

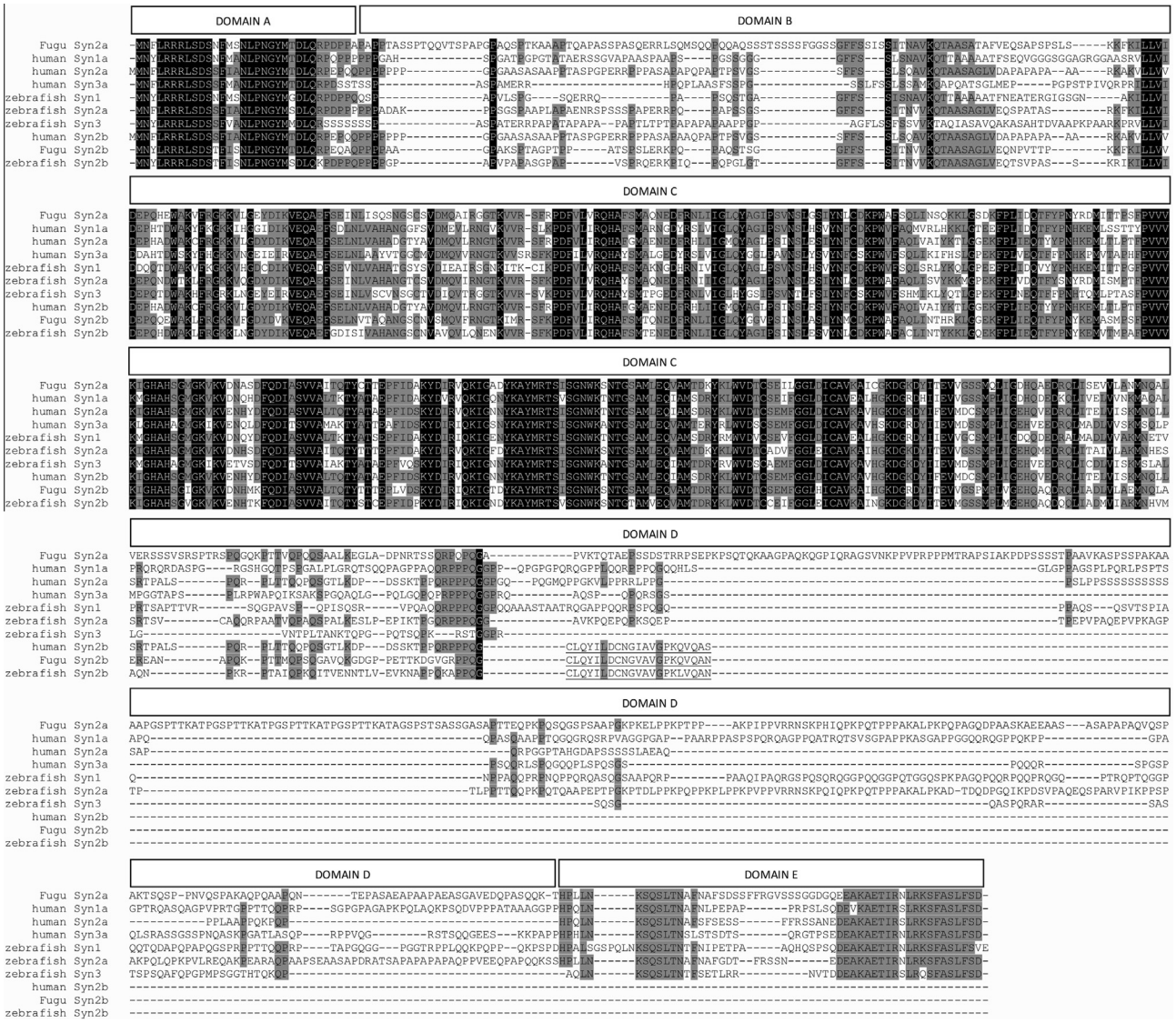

Fig. 1. Multiple sequence alignments of synapsins from zebrafish, Fugu and humans. Identical residues are highlighted in black, and those conserved in at least 50% of sequences in gray. The organization of protein domains is indicated above the sequences. The 21 residues conserved between zebrafish, Fugu and human Syn2b proteins are underlined. Only the Syn2a/b proteins of Fugu are shown.