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Expression of collapsin response mediator proteins in the nervous system of embryonic zebrafish

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Abstract

Collapsin response mediator proteins (CRMPs also known as TUC, Drp, Ulip, TOAD-64) are cytosolic phosphoproteins that are involved in signal transduction during axon growth and in cytoskeletal dynamics. Here we report cloning and mRNA expression patterns of CRMP-1, -2, -3, -4 and, owing to a genome duplication in teleosts, two homologs of CRMP-5 (CRMP-5a and -5b) in embryonic zebrafish at 16 and 24 h post-fertilization (hpf). CRMPs are evolutionarily conserved and zebrafish CRMPs show amino acid identities of 76–90% with their homologs in humans, with the exception of CRMP-3, which shows only 67% homology. Between 16 and 24 hpf, expression of CRMPs generally increased in many regions of the CNS undergoing neuronal differentiation and axonogenesis, but not in the proliferative ventricular zone. Structures that were typically labeled by most, but not all the CRMP probes were the telencephalon, the nucleus of the tract of the post-optic commissure, the epiphysis, the nucleus of the medial longitudinal fascicle, clusters of hindbrain neurons, cranial ganglia, as well as Rohon-Beard neurons. No expression of CRMP mRNAs was observed outside the nervous system. Thus, expression patterns of different CRMP family members correlate with neuronal differentiation and axonogenesis in embryonic zebrafish.

Keywords: Ulip; Drp; TOAD-64; TUC; Semaphorin; *Danio rerio*

1. Results and discussion

Of the five known CRMPs (CRMP-1 to -5) in mammals, CRMP-2 was initially discovered as a signal transducing molecule of the growth cone collapsing activity of semaphorins (Goshima et al., 1995). CRMPs are probably also involved in the signaling activity of other guidance molecules. Beyond a role in axon growth and guidance, CRMPs have been implicated in axon regeneration, neuronal polarity, apoptosis and cell migration in the nervous system (for review see Quinn et al., 1999; Liu and Strittmatter, 2001; Charrier et al., 2003; Arimura et al., 2004).

Zebrafish have emerged as a powerful model system to study nervous system development, due to their external development, transparency of embryos and the availability of mutants, transgenic lines and gene knock-down technology (reviewed in Beattie et al., 2002; Hjorth and Key, 2002; Lewis and Eisen, 2003; Lee and Chien, 2004). To facilitate the analysis of CRMPs in axon growth and neural differentiation in zebrafish we cloned homologs of all five CRMPs in this species and determined their expression patterns at 16 h post-fertilization, when the first neurons undergo axonogenesis, and at 24 hpf, when a simple axonal scaffold is established (Hjorth and Key, 2002).

1.1. Cloning of CRMP genes in zebrafish

To clone CRMP homologs in zebrafish we searched the zebrafish genome and found six predicted CRMP genes. To obtain full-length sequences we used EST clones, containing either full length or partial CRMP homologs, as well as PCR and RACE techniques where necessary. Sequences were confirmed by PCR using cDNA derived from adult zebrafish brains as templates. Zebrafish CRMP genes coded for proteins of 564–575 amino acids in length. For three of the zebrafish genes, BLAST analysis

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A

	zCRMP1	zCRMP2	zCRMP3	zCRMP4	zCRMP5a	zCRMP5b	hCRMP1	hCRMP2	hCRMP3	hCRMP4	hCRMP5
zCRMP1	100	69	59	67	48	49	**79**	71	65	70	50
zCRMP2		100	67	71	49	48	73	**90**	74	74	50
zCRMP3			100	60	43	43	63	***68***	***67***	62	44
zCRMP4				100	47	47	70	73	67	**81**	48
zCRMP5a					100	75	50	50	48	48	**80**
zCRMP5b						100	50	49	47	47	**76**
hCRMP1							100	77	69	74	49
hCRMP2								100	75	76	50
hCRMP3									100	70	48

B

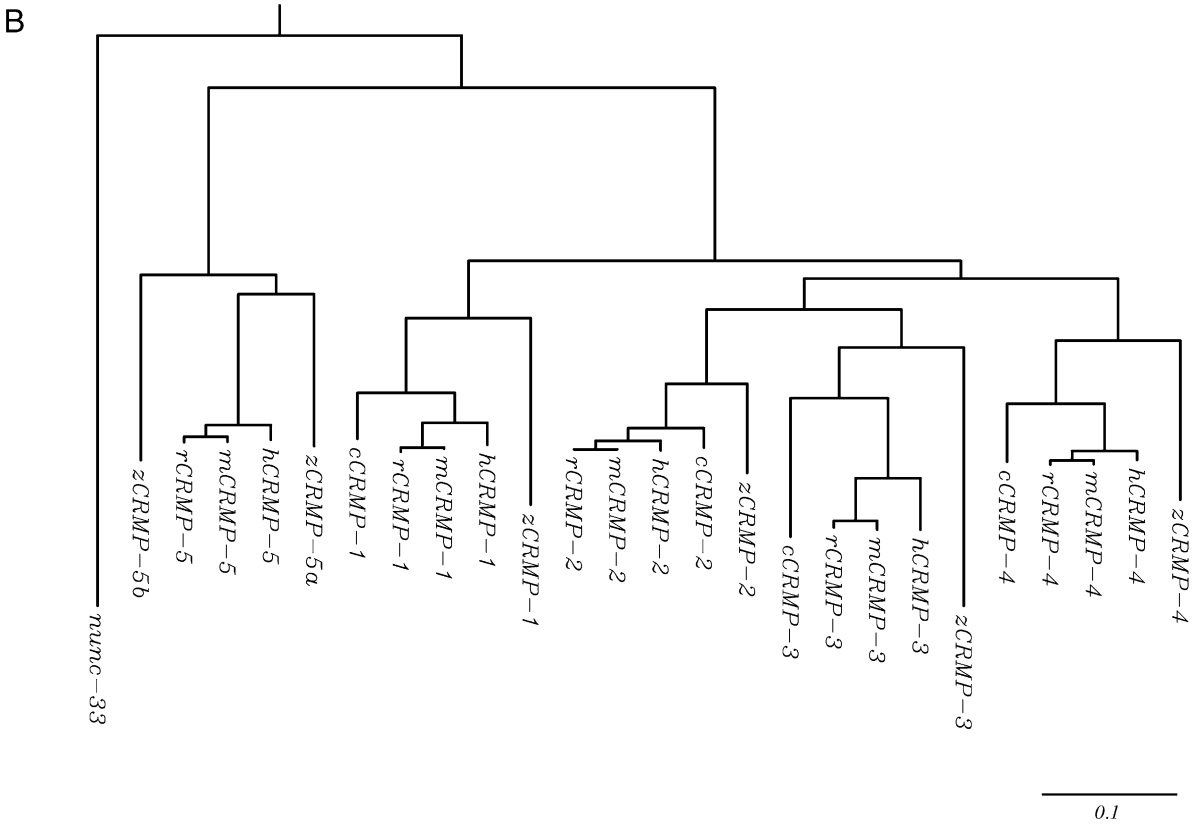

Fig. 1. Comparisons of amino acid sequence identities of CRMPs in different species. (A) Pair-wise comparisons show high percentages of amino acid homologies of zebrafish CRMPs with their human homologs, except for zebrafish CRMP-3, which shows relatively low similarity with human CRMP-2 and -3. (B) Multiple comparisons in a phylogenetic tree group all zebrafish CRMPs with their respective homologs in other vertebrates. Unc-33 (nunc-33), the *C. elegans* homolog of the CRMPs, was added as an outgroup. The scale bar represents 10 substitutions per 100 amino acids.

of the deduced amino acid sequence indicated highest similarities (79–90%, Fig. 1A) with specific human CRMPs, indicating that we found zebrafish CRMP-1 (AY987372) -2 (AY987375) and -4 (AY987373). Two sequences were similarly closely related to human CRMP-5 (76 and 80%). These genes probably arose by a genome duplication in teleost fish (Amores et al., 2004; Chen et al., 2004) and were designated CRMP-5a (AY987371) and CRMP-5b (AY987370), in accordance with the zebrafish nomenclature for duplicated genes (http://zfin.org/zf_info/nomen.html). One sequence was equally closely related to CRMP-2 (68%) and -3 (67%). This homology is markedly lower than that of the other zebrafish molecules to their human homologs. We constructed a phylogenetic tree using the Clustal method (Chenna et al., 2003) with known CRMPs from different vertebrate species and unc-33, the *C. elegans* homolog of CRMPs (Hedgecock et al., 1985), as an outgroup. We found that the zebrafish gene segregated with CRMP-3 (Fig. 1B). Thus, we provisionally identified this gene as zebrafish CRMP-3 (AY987374). All other CRMPs segregated with their respective species homologs, as expected from pair-wise comparisons. A graphical alignment of amino acid sequences of zebrafish CRMPs