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Expression of *rasgef1b* in zebrafish

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Abstract

The *rasgef* genes encode a subgroup of highly conserved Ras guanine nucleotide exchange factors. While EST projects revealed the presence of *rasgef* genes in organisms that range from nematodes to humans, their functions remain to be elucidated. In zebrafish two *rasgef* genes, *rasgef* and *rasgef1b*, have been identified and high throughput analysis revealed tissue specific embryonic expression for *rasgef1b*. Here, we show that three *rasgef1b*-transcripts are generated from two transcriptional start sites and by alternative splicing. Detailed expression analyses show that *rasgef1b* is expressed in a subset of adaxial cells, in the anterior part of somites, in the rostral part of the mid-hindbrain boundary and in the rhombomere boundaries. In the larva, *rasgef1b* is further expressed in the pallium and the inner nuclear layer of the retina. We also find that *rasgef1b* is expressed maternally and that the ubiquitous distribution of maternal transcripts disappears shortly after mid-blastula transition. At early epiboly stages, *rasgef1b* expression is restricted to the margin with low levels of expression on the ventral and high levels of expression on the dorsal side. Finally, we show that early zygotic expression is regulated by Nodal and FGF signals and that these signals have different activities in regulating the level and distribution of early zygotic *rasgef1b* mRNA expression.

Keywords: *rasgef*; *rasgef1b*; Zebrafish; Nodal; FGF; Germring; Somite; Rhombomere boundaries; Mid-hindbrain boundary; Inner nuclear layer

1. Results and discussion

1.1. Characterization of rasgef1b gene in zebrafish

In a screen designed to identify genes regulated by Nodal signaling in zebrafish (details will be described elsewhere), we isolated a cDNA fragment with homology to genes encoding Ras-associated guanine nucleotide exchange factors (RasGEFs). By using this fragment as a probe, a 2.1 kb cDNA was isolated from an embryonic cDNA-library (for details see Section 2). Sequencing of this cDNA clone revealed an open reading frame of 1422 bp as well as 244 bp of 5′ and 368 bp of 3′ untranslated sequences. Blast search comparison of the coding region confirmed that the encoded protein belongs to the family of the highly conserved RasGEF proteins (Ferreira et al., 2002). In common, the RasGEF proteins possess two domains, a highly conserved carboxy-terminal RasGEF domain (Fig. 1a) and a slightly less conserved amino-terminal RasGEFN domain with a yet unknown function. At the level of the protein sequence, vertebrate RasGEF1B proteins display 80–95% identity in the RasGEFN domain and 91–98% identity in the RasGEF domain. RasGEF domains normally activate small GTPases of the Ras/Rho family by catalyzing the exchange of the inactive GDP-bound form to the activated GTP-bound form (Quilliam et al., 2002). Whether or not RasGEF1B proteins have a similar function remains to be investigated, particularly because their GEF domains display characteristic differences when compared to the GEF domains of the related Ras activating proteins CDC25 and SOS (Fig. 1a) (Jones et al., 1991; Chardin et al., 1993). These conserved changes include sequence variations and two insertions of 6–7 amino acids (indicated by red boxes in Fig. 1a). Currently, the only evidence for a biological function of

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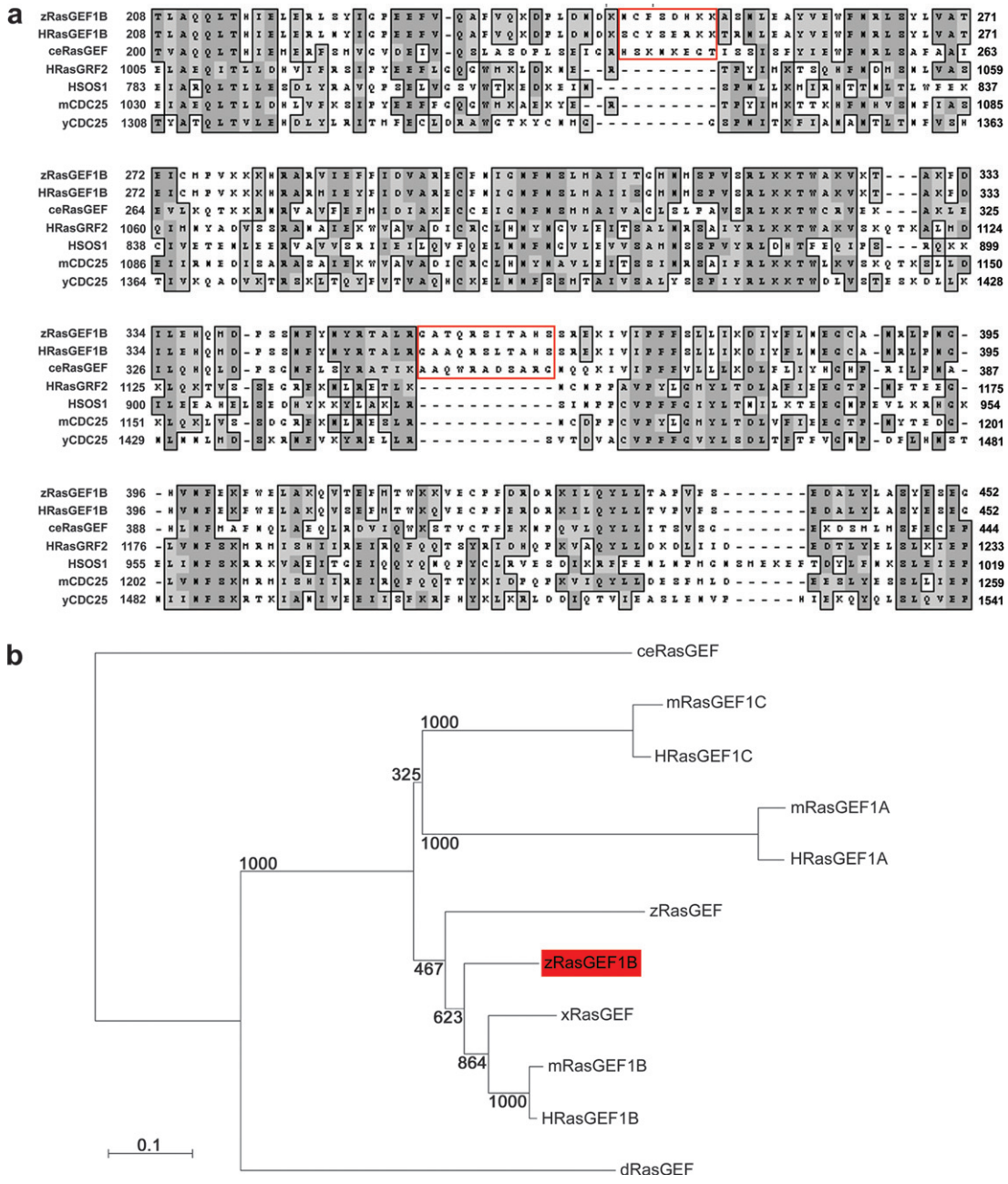

Fig. 1. RasGEF proteins are a highly conserved subgroup of Ras guanine nucleotide exchange factors. (a) Multiple amino acid alignment of solely the RasGEF domains from zebrafish RasGEF1B (zRasGEF1B), human RasGEF1B (HRasGEF1B), *Caenorhabditis elegans* (ceRasGEF), human Ras guanine nucleotide releasing factor 2 (HRasGRF2), human SOS1 (HSOS1), mouse CDC25 (mCDC25) and yeast CDC25 (yCDC25). Red boxes indicate two insertions of 6–7 amino acids of RasGEF1B proteins. Identical and similar amino acids are indicated by dark and light gray backgrounds, respectively. The numbers refer to the position in the amino acid sequence. (b) Phylogenetic analysis of RasGEF-related proteins from vertebrates and invertebrates. In vertebrates three RasGEF proteins can be distinguished with the subgroup of RasGEF1B proteins showing the highest homology to the zebrafish gene *rasgef1b*. Numbers are bootstrap values for 1.000 trials. Accession codes: zebrafish (zRasGEF1B: NP_956123, zRasGEF: NP_001003479), human (HRasGEF1A: AAH22548, HRasGEF1B: NP_689758, HRasGEF1C: AAH36802, HRasGRF2: AAB80953, HSOS1: AAM22406), mouse (mRasGEF1A: XP_982035, mRasGEF1B: AAH50858, mRasGEF1C: NP_083280, mCDC25: NP_035375), *Xenopus tropicalis* (xRasGEF: NP_001016913), *Drosophila melanogaster* (dRasGEF: AAL49083), *Caenorhabditis elegans* (ceRasGEF: AAB00628), and *Saccharomyces cerevisiae* (yCDC25: Q02342).

RasGEF1B proteins comes from studies in invertebrates. *Drosophila* (Kiger et al., 2003) and *Caenorhabditis elegans* (www.wormbase.org/db/gene/gene?name = WBGene 00019902; class = Gene) both contain a single *rasgef1b*-related gene, named *rasgef*, which shows 35% and 36–37% identity to the genes encoding the vertebrate RasGEF1B proteins, respectively. In large scale RNAi experiments, knock down of *Drosophila* RasGEF in Schneider-cells was found to result in a slight change in cell morphology. However, no defects were found when *Drosophila* or *C. elegans rasgef* were knocked down in the entire organism (Kamath et al., 2003; Rual et al., 2004; Sonnichsen et al., 2005).