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Developmental expression of the *slurp-like1/ly2.3/ly97.3* and *slurp-like2/ly2.2/ly97.2* genes during zebrafish early embryogenesis

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ABSTRACT

Mammalian SLURP1 and SLURP2 belong to the Ly-6/uPAR superfamily and are involved in maintaining the physiological integrity of keratinocytes. However, the developmental expression and functions of other Ly-6/uPAR family genes in vertebrates are still obscure. We have isolated novel Ly-6/uPAR family genes *slurp-like1* (*ly2.3/ly97.3*) and *slurp-like2* (*ly2.2/ly97.2*) in zebrafish. Both the Slurp-like1 and Slurp-like2 proteins contain the typical signal sequence and carboxy-terminal CCXXXXCN (X: an arbitrary amino acid) consensus sequence of the Ly-6/uPAR family but lack a transmembrane domain and a GPI-anchoring signal sequence, suggesting that both proteins may function as secretory proteins. Whole-mount *in situ* hybridization analysis revealed that *slurp-like1* was predominantly expressed in the floor plate of the neural tube and in the hypochord of the notochord at 24 h post-fertilization (hpf) and detected in the liver and intestinal bulb at 72 hpf, while *slurp-like2* was expressed in the midbrain and hindbrain at 24 hpf and detected in the liver and pancreas at 72 hpf. Differential expression profiles of the *slurp-like1* and *slurp-like2* genes suggest the distinct physiological involvement of these genes in zebrafish early embryogenesis.

1. Results and discussion

1.1. Isolation of two slurp-like genes in zebrafish

Recent genomic studies and RNA sequence analysis have suggested that uncharacterized small proteins translated from short open reading frames exist in the zebrafish genome (Pauli et al., 2014). Using whole-mount *in situ* hybridization (WISH) screening of these genes, we found that both the *secreted mammalian Ly-6/uPAR-related protein* (*slurp*)*-like1* and *slurp-like2* genes exhibit unique molecular characterizations and developmental expression patterns during zebrafish embryogenesis as described below. Zebrafish *slurp-like1* and *slurp-like2* genes encode 94 and 95 amino acids, respectively, containing the Ly-6/uPAR domain characterized by 10 conserved cysteine residues (Fig. 1A: asterisks) (Vasilyeva et al., 2017). Importantly, the carboxy-terminal consensus motif CCXXXXCN (X: an arbitrary amino acid) was highly conserved between the mammalian SLURP proteins and zebrafish Slurp-like proteins (Fig. 1A: black bar). Both Slurp-like1 and Slurp-like2 possess a typical signal sequence predicted by the SignalP algorithm but lack a transmembrane domain and a GPI-anchoring signal sequence, suggesting that both gene products encode novel secretory proteins. Wang et al. have recently reported zebrafish *Ly-6/uPAR* family genes *ly2.2/ly97.2* and *ly2.3/ly97.3* (Wang et al., 2016); these genes are identical to *slurp-like2* and *slurp-like1*, respectively (Supplemental Fig. 1). The authors suggest that both genes have a role in innate immunity. As described below, our results for developmental expression patterns of *slurp-like1* and *slurp-like2* during early zebrafish embryogenesis suggest the involvement of these genes in various morphological processes. In mammals, SLURP1 and SLURP2 are generated by the keratinocytes comprising the mucocutaneous epithelium (Arredondo et al., 2005, 2006). Accumulative evidence demonstrates that the secreted SLURP1 and SLURP2 proteins function as autocrine and paracrine factors regulating the growth and differentiation of the keratinocytes (Arredondo et al., 2007; Kawashima et al., 2012), suggesting that the SLURP proteins play important roles in regulating epithelial cells. Interestingly, point mutations in human *SLURP1* cause an autosomal inflammatory disorder of the skin called Mal de Meleda (Fischer et al., 2001; Martina et al., 2003). Phylogenic analysis based on the Slurp-like1 and Slurp-like2 primary protein structures suggests close relationship to mammalian SLURP1 and SLURP2 (Fig. 1B). Therefore, we have examined the developmental expression profiles of *slurp-like1/ly2.3/ly97.3* and *slurp-like2/ly2.2/ly97.2* genes during early zebrafish embryogenesis.

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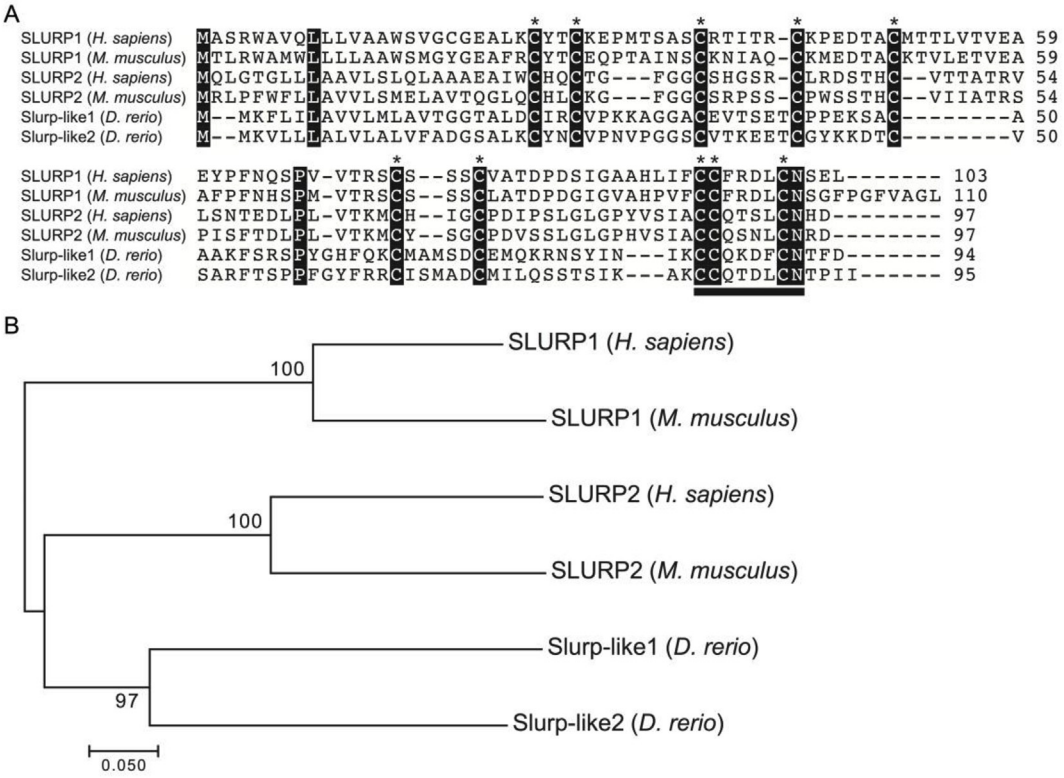

Fig. 1. Amino acid alignment and phylogenic tree of Slurp proteins. (A) Amino acid sequence alignment of Slurp proteins. Slurp proteins such as human SLURP1 (AAT01436) and SLURP2 (AAT00512), mouse SLURP1 (NP_065265) and SLURP2 (NP_001075430), and zebrafish Slurp-like1 (LC378548) and Slurp-like2 (LC378549) were aligned using the ClustalW multiple alignment program. Conserved amino acids are highlighted in black, while conserved cysteine residues are indicated with asterisks. Black bar indicates the position of carboxy-terminal consensus motif CCXXXXCN (X: an arbitrary amino acid). (B) Phylogenetic tree of Slurp proteins. The evolutionary distances were computed using the p-distance method and are in the units of the number of amino acid differences per site. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2018).

1.2. Temporal expression of slurp-like1 and slurp-like2 transcripts

Total RNA was isolated from embryos at various developmental stages and the temporal expression profiles of *slurp-like1/ly2.3/ly97.3* and *slurp-like2/ly2.2/ly97.2* transcripts were examined by reverse transcription (RT)-PCR (Fig. 2). The *slurp-like1* transcript was marginal in the 2-cell stage (Fig. 2). The expression of *slurp-like1* gradually increased during the 2-cell, dome, shield and bud stages, and its expression levels were maintained in the 5-somite, 10-somite, 15-somite, 20-somite, 24 h post-fertilization (hpf), 48 hpf and 72 hpf stages (Fig. 2). The *slurp-like2* transcript was marginal until the 20-somite stage (Fig. 2) and was detected in 24 hpf, 48 hpf and 72 hpf stages.

1.3. Expression of slurp-like1 during zebrafish embryogenesis

In mammals, both the *SLURP1* and *SLURP2* genes are expressed in the epithelial cells including the keratinocytes (Arredondo et al., 2005, 2006). Wild-type embryos at different developmental stages were fixed, and the expression of the *slurp-like1* gene was examined by WISH using an antisense *slurp-like1* probe. The expression of *slurp-like1* was very weak in the 1-cell stage (Fig. 3A). Weak and broad *slurp-like1* expression were observed in the embryos at the dome, shield and bud stages (Fig. 3B–D). Expression of *slurp-like1* in deep cells and the yolk edge was detected at the 5-somite, 10-somite, 15-somite and 20-somite stages (Fig. 3E–H). In fact, a transverse section of a *slurp-like1*-stained embryo showed that *slurp-like1* gene was weakly expressed in both the endoderm and yolk syncytial layer (YSL) at the 5-somite stage (Supplemental Fig. 2). At 24 hpf, the *slurp-like1* gene was predominantly expressed in the floor plate (arrow) of the neural tube and in the hypochord (arrowhead) of the notochord (Fig. 3I and J). These expression patterns are apparently unique because such expression profiles of Ly-6/uPAR family genes have not been reported in any mammals or fish (Arredondo et al., 2005; Moriwaki et al., 2009; Wang et al., 2016). At the 48 hpf and 72 hpf stages, *slurp-like1* expression was detected in the liver (red arrowhead) and intestinal bulb (blue arrowhead) (Fig. 3M). Although the *SLURP1* gene in mammals was expressed in keratinocytes of the skin and in immune cells (Arredondo et al., 2005, 2006), the *slurp-like1* gene in zebrafish early embryogenesis is expressed in some of the midline structures such as the floor plate and hypochord and in some of the digestive organs including the liver and intestinal bulb.

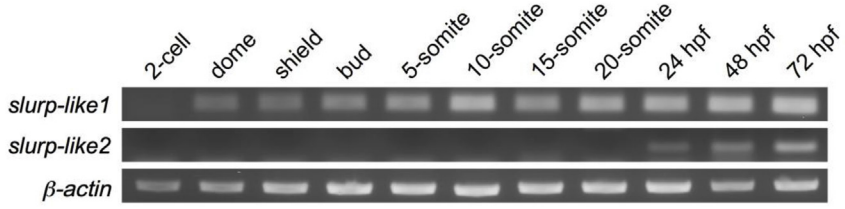

Fig. 2. Temporal expression pattern of *slurp-like* genes during zebrafish early development. Total RNA was isolated from the embryos at the indicated stages, and the transcripts of *slurp-like* genes were amplified by reverse transcription (RT)-PCR. The upper and middle panels exhibit the results of *slurp-like1* and *slurp-like2*, respectively. The bottom panel shows the results of *β-actin* as a control. Similar results were obtained in three independent experiments.