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Gene expression pattern

Cloning and expression pattern of the lysozyme C gene in zebrafish ☆

Feng Liu, Zilong Wen*

Laboratory of Molecular Immunology, Institute of Molecular Agrobiology, 1 Research Link, Singapore, Singapore 117604

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Abstract

Here, we report isolation and developmental expression pattern of the zebrafish lysozyme C gene. Amino acid sequence analysis showed that the zebrafish lysozyme C protein shared ~37–80% identities with the mouse, human, chicken, and carp counterparts. Whole-mount in situ hybridization showed that the lysozyme C gene was expressed in macrophages, as its expression was co-localized with the known myeloid lineage markers L-plastin and PU.1. At 20 hours postfertilization (hpf), most of the lysozyme C positive cells were localized in the yolksac and head mesenchyme but not in the intermediate cell mass, supporting the notion that the primitive macrophage originated from the yolksac (Development 126 (1999) 3735). At 36 hpf, the lysozyme C positive cells scattered within the head and yolksac, and began to appear in the caudal part of axial vein. By 6 days postfertilization (dpf), the lysozyme C positive cells accumulated in the kidney where hematopoiesis had been indicated to take place after 4 dpf (Dev. Dyn. 214 (1999) 323). Taken together, our results demonstrate that the lysozyme C gene is specifically expressed in myeloid lineage, suggesting that it could serve as an excellent marker for genetic screening of both primitive and definitive myeloid lineage development in zebrafish.

Keywords: Zebrafish; Lysozyme C; L-plastin; PU.1; Macrophage; Yolksac; Intermediate cell mass; Kidney; Hematopoiesis

1. Results and discussion

1.1. Cloning and sequence analysis of zebrafish lysozyme C

To study myeloid lineage development using zebrafish as a model system, we searched for myeloid-specific genes by differential screening of zebrafish kidney subtractive cDNA library (data not shown). One of the positive clones we obtained was a 600 bp long cDNA fragment that encodes a 151 aa polypeptide (Fig. 1A). Protein sequence analysis showed that the polypeptide contained an α-lactalbumin/lysozyme C signature motif (Nitta and Sugai, 1989; Fujiki et al., 2000), and was highly similar to the carp (80% identity), chicken (44% identity), mouse (~39% identity), and human (39% identity) lysozyme C (Fig. 1B). We designated this polypeptide as zebrafish lysozyme C. The C-type lysozymes are classified into two different subfamilies, i.e. the calcium binding and non-calcium binding families. Since it lacks an aspartic acid residue at positions 101, 106, and 107 that have been shown to be necessary for calcium binding (Nitta and Sugai, 1989), the zebrafish lysozyme C appears, like the carp and chicken lysozyme C, to belong to the non-calcium binding family. In mouse, there are two highly conserved (~91% identity) lysozyme C genes, M and P, due to gene duplication. The M gene expression is restricted to myeloid lineage, whereas the P gene is expressed only in small intestine (Cross et al., 1988). We thus tested if the lysozyme C gene was duplicated in zebrafish. Southern blot analysis of the zebrafish genomic DNA digested with *E*coRI, *B*amHI, *E*coRV, and *H*indIII, detected a single band which specifically hybridized to the zebrafish lysozyme C cDNA probe, indicating a single copy of the lysozyme C gene in the zebrafish genome (data not shown).

1.2. Lysozyme C is specifically expressed in the macrophage lineage

Reverse transcriptase polymerase chain reaction (RT-PCR) and Northern blot analysis showed that the lysozyme C expression began at 20 hpf, and was predominately restricted in kidney, the main hematopoietic organ, in adult zebrafish (data not shown; Willett et al., 1999; Paw and Zon, 2000). Since the lysozyme C gene is a myeloid lineage-specific marker in higher vertebrate (Faust et al., 2000; Shepard and Zon, 2000), we further tested if it was specifically expressed in the myeloid lineage in zebrafish by whole-mount in situ hybridization. As shown in Fig. 2,

☆ GenBank accession number of the zebrafish lysozyme C is AF402599.

* Corresponding author. Tel.: +65-872-7465; fax: +65-872-7007.
E-mail address: zilong@ima.org.sg (Z. Wen).

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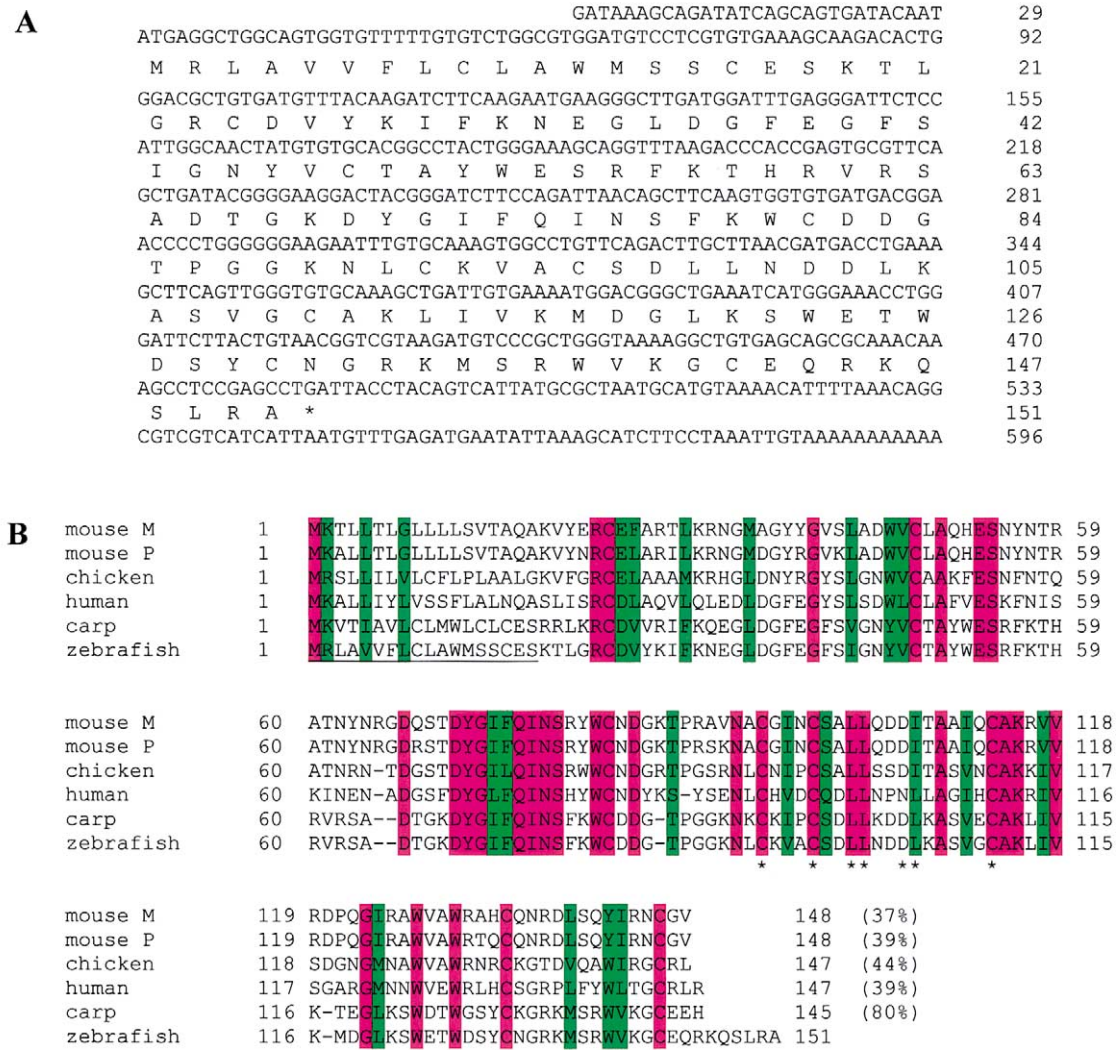

Fig. 1. Sequence analysis of the zebrafish lysozyme C gene. (A) Nucleotide and deducted amino acid sequences of the zebrafish lysozyme C gene. Amino acid is shown as a single letter code and asterisk indicates termination codon. (B) Protein sequence alignment of zebrafish lysozyme C with mouse, human, chicken, and carp homologues. The predicted signal sequence is underlined. The identical and conserved residues were shaded with red and green colors, respectively. Asterisk indicates the α-lactalbumin/lysozyme C signature motif. The percentage at the end of each sequence represents amino acid identities of other vertebrate proteins to that of zebrafish lysozyme C. GenBank accession numbers of these genes are as follows: zebrafish lysozyme C, AF402599; carp lysozyme C, BAA95698; chicken lysozyme C, P00698; human lysozyme C, P00695; mouse lysozyme M, NP-059068; and mouse lysozyme P, NP-038618.

the lysozyme C gene started to express at 20 hpf (Fig. 2A). At this stage until 24 hpf, the lysozyme C positive cells were accumulated, usually as three to four cells together, in the anterior part of the yolksac and head mesenchyme (Fig. 2A, B). At 30 hpf, the positive cells scattered around the head and yolksac, and began to appear in the caudal part of the axial vein and surrounding mesenchyme (~15 cells), where the positive cells reached to ~50 cells by 48 hpf (Fig. 2C–F). By 72 hpf, the lysozyme C positive cells were almost undetectable (data not shown) and then reappeared at 6 dpf in the head kidney (Fig. 2G, H), where the main hematopoiesis takes place at late stages of zebrafish development till adulthood (Willett et al., 1999; Paw and Zon, 2000). Thus, the expression pattern of lysozyme C was very similar to that of the two known myeloid lineage markers, L-plastin and C/EBP1, in zebrafish (Herbomel et al., 1999, 2001; Lyons et al., 2001) (Fig. 3A, B), indicating that these lysozyme C positive cells were likely macrophages. To confirm this, double in situ hybridization was carried out with both lysozyme C and L-plastin, showing that the lysozyme C expression was co-localized with L-plastin in the same cells (Fig. 3C, D). Similarly, the lysozyme C was also co-expressed with PU.1, another early myeloid lineage marker (Fig. 3E–H) (Bennett et al., 2001), but not with GATA1, an erythroid lineage marker (Detrich et al., 1995) (Fig. 3I). These results demonstrate that the lysozyme C positive cells are indeed macrophages.

In conclusion, we have isolated the zebrafish lysozyme C

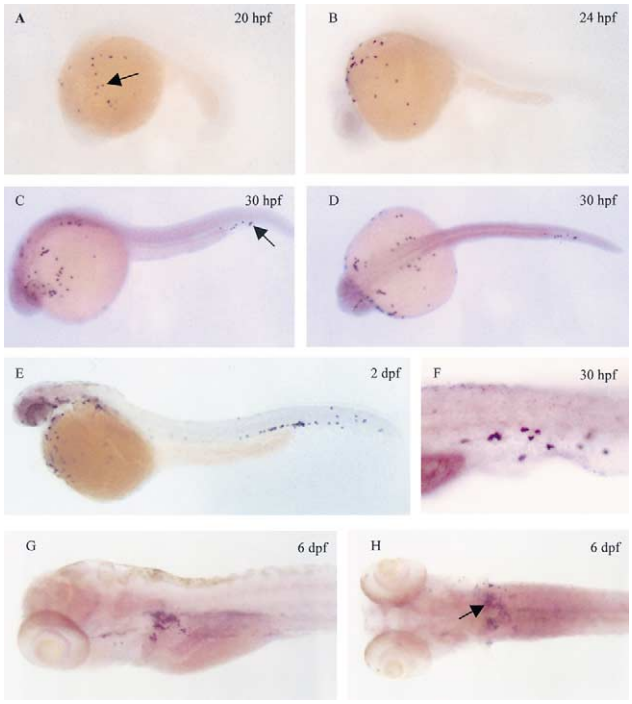

Fig. 2. Expression pattern of the lysozyme C gene by whole-mount in situ hybridization during embryogenesis. Whole-mount in situ hybridization with digoxigenin-labeled lysozyme C probe was carried out according to standard protocol (Westerfield, 1995). Lateral view of 20 hpf (A), 24 hpf (B), 30 hpf (C,F), and 48 hpf embryos (E) are shown whereas (D) is a dorsal view of 30 hpf embryo. Lateral view (G) and dorsal view (H) of 6 dpf embryos are shown. Arrows in panels A,C,H indicate lysozyme positive cells in the yolksac, caudal part of the axial vein, and pronephros, respectively. In (C,D,E) differently focused pictures were fused electronically to show the lysozyme C positive cells in the yolksac and tail region.

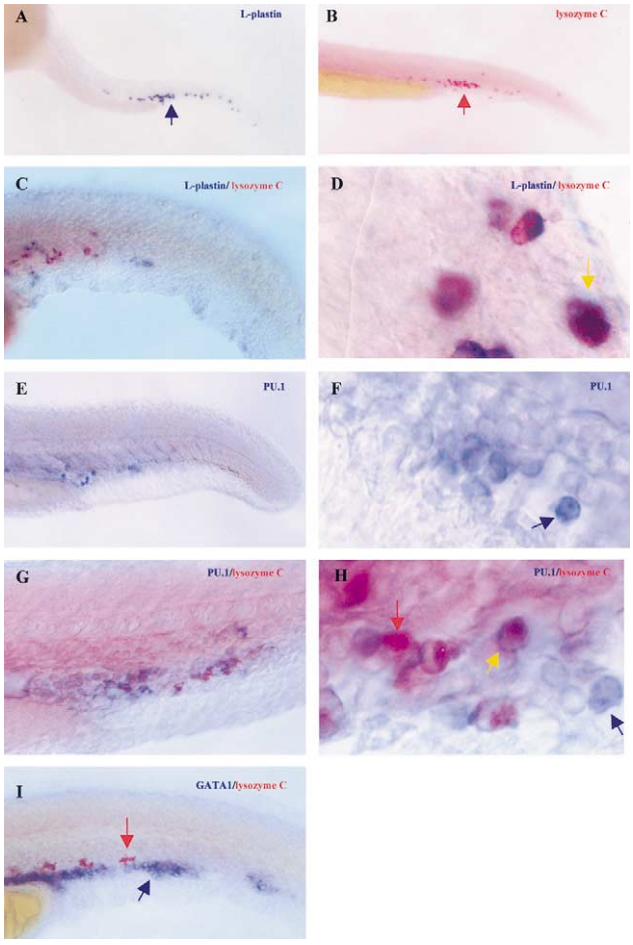

Fig. 3. Macrophage-specific expression of lysozyme C by double in situ hybridization. (A,B,E,F) Lateral view of posterior part of 36 hpf embryo stained with digoxigenin-labeled L-plastin (blue), fluorescein-labeled lysozyme C (red), and digoxigenin-labeled PU.1 (blue), respectively. (C,D,G,H) Double in situ hybridization using fluorescein-labeled lysozyme C (red) together with digoxigenin-labeled either L-plastin (C,D) or PU.1 (G,H). (I) Double in situ hybridization with the lysozyme C (red) and GATA1 (blue). The yellow arrow represents the double positive cells whereas the red and blue arrows indicate single positive cells. Magnification in panels D,F,H is 100× and in panel G is 40×.

gene and have shown that it is specifically expressed in macrophages. Thus, the lysozyme C gene may serve as an excellent marker for studying myeloid lineage development in zebrafish.

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