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Expression patterns of *dnmt3aa*, *dnmt3ab*, and *dnmt4* during development and fin regeneration in zebrafish

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ABSTRACT

Epigenetic modifications such as DNA methylation and chromatin modifications are critical for regulation of spatiotemporal gene expression during development. In mammals, the *de novo*-type DNA methyltransferases (Dnmts), Dnmt3a and Dnmt3b, are responsible for the creation of DNA methylation patterns during development. In addition to developmental processes, we recently showed that DNA methylation levels are dynamically changed during zebrafish fin regeneration, suggesting that the *de novo*-type Dnmts might play roles in the regulation of gene expression during regeneration processes. Here, we showed the detailed expression profiles of three zebrafish *dnmt* genes (*dnmt3aa*, *dnmt3ab*, and *dnmt4*), which were identified as the orthologues of mammalian *dnmt3a* and *dnmt3b*, during embryonic and larval development, as well as fin regeneration processes. *dnmt3aa* and *dnmt3ab* are expressed in the brain, pharyngeal arches, pectoral fin buds, intestine, and swim bladder; the specific expression of *dnmt3aa* is observed in the pronephric duct during larval development. *dnmt4* expression is observed in the zona limitans intrathalamica, midbrain–hindbrain boundary, ciliary marginal zone, pharyngeal arches, auditory capsule, pectoral fin buds, intestine, pancreas, liver, and hematopoietic cells in the aorta–gonad–mesonephros and caudal hematopoietic tissue from 48 to 72 h post-fertilization. Furthermore, during fin regeneration, strong *dnmt3aa* expression, and faint *dnmt3ab* and *dnmt4* expression are detected in blastema cells at 72 h post-amputation. Taken together, our results suggest that zebrafish Dnmt3aa, Dnmt3ab, and Dnmt4 may play roles in the formation of various organs, such as the brain, kidney, digestive organs, and/or hematopoietic cells, as well as in the differentiation of blastema cells.

1. Results and discussion

In vertebrates, spatially and temporally regulated gene expression is required for cell proliferation and differentiation during development, and one of the critical factors for the regulation of gene expression is epigenetic modifications including DNA methylation and chromatin modifications (Turek-Plewa and Jagodziński, 2005; Mellor et al., 2008; Bogdanović et al., 2012; Smith and Meissner, 2013). In mammals, cytosines in CpG dinucleotides are predominately methylated at the 5 position by 3 DNA methyltransferases (Dnmt1, Dnmt3a, and Dnmt3b) (Goll and Bestor, 2005; Jones, 2012). Genetic and biochemical evidence in mice have revealed that although Dnmt1 mainly functions in the maintenance of pre-existing DNA methylation (Li et al., 1992; Lei et al., 1996), Dnmt3a and Dnmt3b contribute to the establishment of *de novo* DNA methylation patterns during development (Okano et al., 1999). Two *de novo* Dnmt proteins, Dnmt3a and Dnmt3b, showed distinct expression patterns during mouse development; Dnmt3a is ubiquitously expressed after E10.5, whereas Dnmt3b is specifically detected in progenitor cells during hematopoiesis, spermatogenesis, and neurogenesis (Okano et al., 1999; Watanabe et al., 2004; Watanabe et al., 2006). These distinct expression patterns result in different developmental defects; Dnmt3a-deficient mice normally develop to term, but die about 4 weeks after birth (Okano et al., 1999); Dnmt3b-deficient mice show an embryonic lethal phenotype with multiple developmental defects (e.g. rostral neural tube defects, liver hypotrophy, ventricular septal defect, and haemorrhage) (Okano et al., 1999; Ueda et al., 2006); Missense mutations in *Dnmt3b* cause similar phenotypes leading to patients with immunodeficiency-centromeric instability-facial anomalies (ICF) syndrome (Ueda et al., 2006). These results indicate that these two genes have non-overlapping functions. In addition to the developmental stages, recent reports have revealed that Dnmt3a play roles in somatic stem cells; Dnmt3a regulates the expression of neurogenic genes in postnatal neural stem cells (Wu et al., 2010); Dnmt3a

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is essential for differentiation, but not for self-renewal, of hematopoietic stem cell (HSCs) (Challen et al., 2012).

In contrast to mammals, 8 zebrafish *Dnmt* genes were cloned and phylogenetic analyses revealed that 6 of them [*dnmt3* (also known as *dnmt3b3*), *dnmt3aa* (also known as *dnmt8*, *dnmt3a2*), *dnmt3ab* (also known as *dnmt6*, *dnmt3a1*), *dnmt3b* (also known as *dnmt7*, *dnmt3b2*), *dnmt4* (also known as *dnmt3b1*), and *dnmt5* (also known as *dnmt3b4*)] are orthologues of mammalian *Dnmt3a* and *Dnmt3b* (Xie et al., 1999; Shimoda et al., 2005; Campos et al., 2012). We have previously reported that expression of *dnmt3b* is ubiquitous during zebrafish development (Shimoda et al. 2005). Despite partial gene expression analyses by *in situ* hybridization and real-time PCR (Smith et al., 2005; Rai et al., 2010; Smith et al., 2011; Campos et al., 2012), detailed spatiotemporal expression patterns of *dnmt3*, *dnmt3aa*, *dnmt3ab*, *dnmt4*, and *dnmt5* have not yet been reported. We examined the expression patterns of these 5 genes, and found that 3 of them (*dnmt3aa*, *dnmt3ab*, and *dnmt4*) show tissue-specific expression patterns during development (data not shown). In addition, we recently found that the DNA methylation levels (5-methyl cytosine, 5mC and 5-hydroxymethyl cytosine, 5hmC) are transiently reduced at the early stages of fin regeneration (36 h post amputation, hpa), and these levels of 5mC and 5hmC are gradually recovered in the course of fin regeneration (Hirose et al., 2013). Based on our results, we hypothesized that some *Dnmt* genes might be expressed in fin regenerate and could function in fin regeneration processes possibly through the regulation of gene expression. Thus, in this study, we analyzed the spatiotemporal expression of *dnm3aa*, *dnmt3ab*, and *dnmt4* genes during embryonic and larval development and during fin regeneration in zebrafish.

1.1. dnmt3aa expression during zebrafish development

As previously reported, phylogenetic analysis have revealed that Dnmt3aa and Dnmt3ab are closely related to mammalian Dnmt3a, while Dnmt4 is closely related to mammalian Dnmt3b (Shimoda et al., 2005; Campos et al., 2012). To analyze the expression pattern of these *dnmt3*-related genes, we first examined the spatiotemporal expression of *dnmt3aa* during embryonic and larval development of zebrafish by whole-mount *in situ* hybridization. A previous report showed that the maternal transcripts of *dnmt3aa* and *dnmt3ab* can be detected and the expression of these genes is upregulated following the midblastula transition (MBT) (Smith et al., 2011). After the MBT, faint and ubiquitous expression of *dnmt3aa* was observed in whole embryos (data not shown). At the 10 somite (s) stage, *dnmt3aa* began to be expressed in the intermediate mesoderm fated to become the pronephric epithelia as a pair of bilateral stripes (Fig. 1A and B). *dnmt3aa* expression in the pronephric duct (pd), neural tube, and somitic mesoderm was evident at the 18s stage (Fig. 1C and D), and the expression,

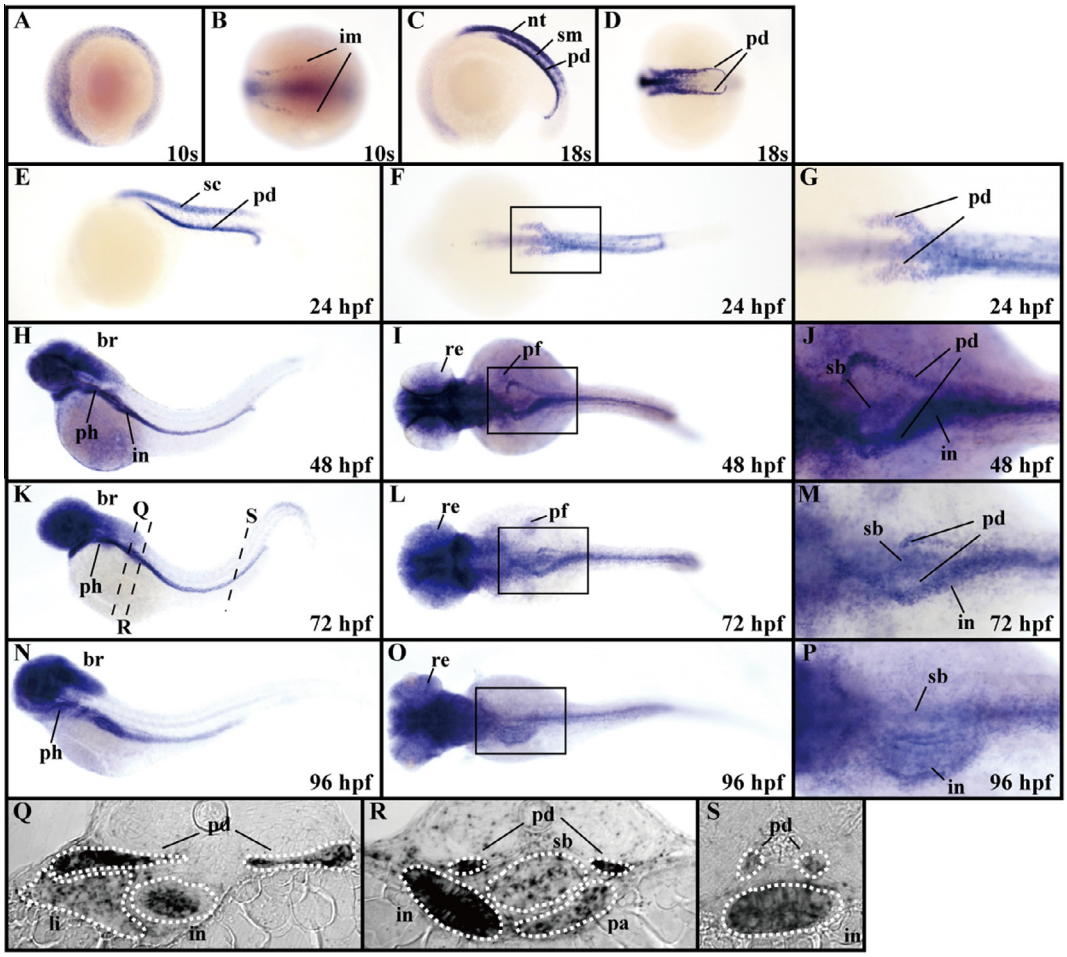

Fig. 1. Expression patterns of *dnmt3aa* in zebrafish embryos and larvae. (A–P) *dnmt3aa* expression was examined by whole-mount *in situ* hybridization at the 10s and 18s stages as well as at 24, 48, 72, and 96 hpf. Lateral views, anterior to the left (A, C, E, H, K, and N). Vegetal pole views of the tail-bud region, anterior to the left (B and D). Dorsal views, anterior to the left (F, I, L, and O). The boxed areas in (F), (I), (L), and (O) are shown enlarged in (G), (J), (M), and (P), respectively. (Q–S) Transverse sections showing *dnmt3aa* expression. The transverse sections were cut at the levels indicated by the dashed black lines in (K). br, brain; im, intermediate mesoderm; in, intestine; li, liver; nt, neural tube; pa, pancreas; pd, pronephric duct; pf, pectoral fin buds; ph, pharyngeal arches; re, retina; sb, swim bladder; sc, spinal cord; sm, somitic mesoderm.