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A novel gene, *Ami* is expressed in vascular tissue in *Xenopus laevis*

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Abstract

We report the isolation and expression pattern of a novel gene, *Ami* in *Xenopus laevis*. *Ami* was initially isolated as a highly expressed gene in cardiovascular tissues. The deduced amino acid sequence of Ami was most closely similar to human complement factor D and mouse adipsin in mammals. In adult *Xenopus* tissues, the transcript of *Ami* was detected in liver, fat body, lung, gut, vessel, heart, muscle, testis, and ovary, but expression in blood cells or skin was hardly detected. This expression profile was significantly different from that observed for mammalian homologues. *Ami* transcripts in *Xenopus laevis* were expressed from the late neurula stage, remained constant until the tadpole stage. The mRNA localized to paraxial regions at the neurula stage and anterior ventral regions at the tailbud stage. From the late tailbud to tadpole stage, expression was detected along the forming blood vessels, including the anterior cardinal veins, posterior cardinal veins, intersomitic veins, dorsal longitudinal anastomosing vessel, dorsal aorta, pronephric sinus, and most prominently around the vascular vitelline network. The expression around the vascular vitelline network demonstrated left–right asymmetry in stage 42 embryo. Comparison with the endothelium marker, *Xmsr*, suggested that *Ami* is expressed in endothelial cells.

Keywords: Adipsin; Vessel; Vein; Endothelium; Notochord; Complement factor; Left–right asymmetry; *Xmsr*; Anterior cardinal vein; Posterior cardinal vein; Intersomitic veins; Dorsal longitudinal anastomosing vessel; Dorsal aorta; Pronephric sinus; Vascular vitelline network

1. Results and discussion

The cardiovascular system is one of the first organs to function in the developing embryo. It consists of endothelial cells lining the vascular tubes, cardiac and smooth muscles cells, and pericytes enfolding the endothelial cells. The system and the components not only provide the nutrients and oxygen to tissues, but also has important roles in the development of other organs, such as kidney, liver, and pancreas (Nikolova and Lammert, 2003). Despite numerous and extensive studies, the precise mechanisms of cardiovascular development are not fully understood. To address this, we undertook a differential screening to identify genes with possible functional involvement in the developing cardiovasculature.

1.1. Isolation of Ami

During the screening for genes expressed in cardiovascular tissues, we found a clone that showed strong expression in developing vascular tissue. We performed 5′ and 3′ RACE and obtained the complete sequence of the transcript (Fig. 1A, GenBank Accession No. AB238233), which was 985 base pairs long and encoded a putative protein of 263 amino acid residues. We named this gene "*Ami*" ("mesh" in Japanese), according to the mRNA expression pattern. The putative amino acid sequence of Ami was most similar to human complement factor D (CFAD) protein. CFAD is a protease that specifically cleaves a lysine-arginine bond of complement factor B in human serum (Lesavre et al., 1979; Niemann et al., 1984). CFAD is also an orthologue of mouse adipsin, a gene involved in adipose differentiation (Cook et al., 1985; White et al., 1992). A comparison of the amino acid sequences of Ami and its homologous sequences of several species are shown in

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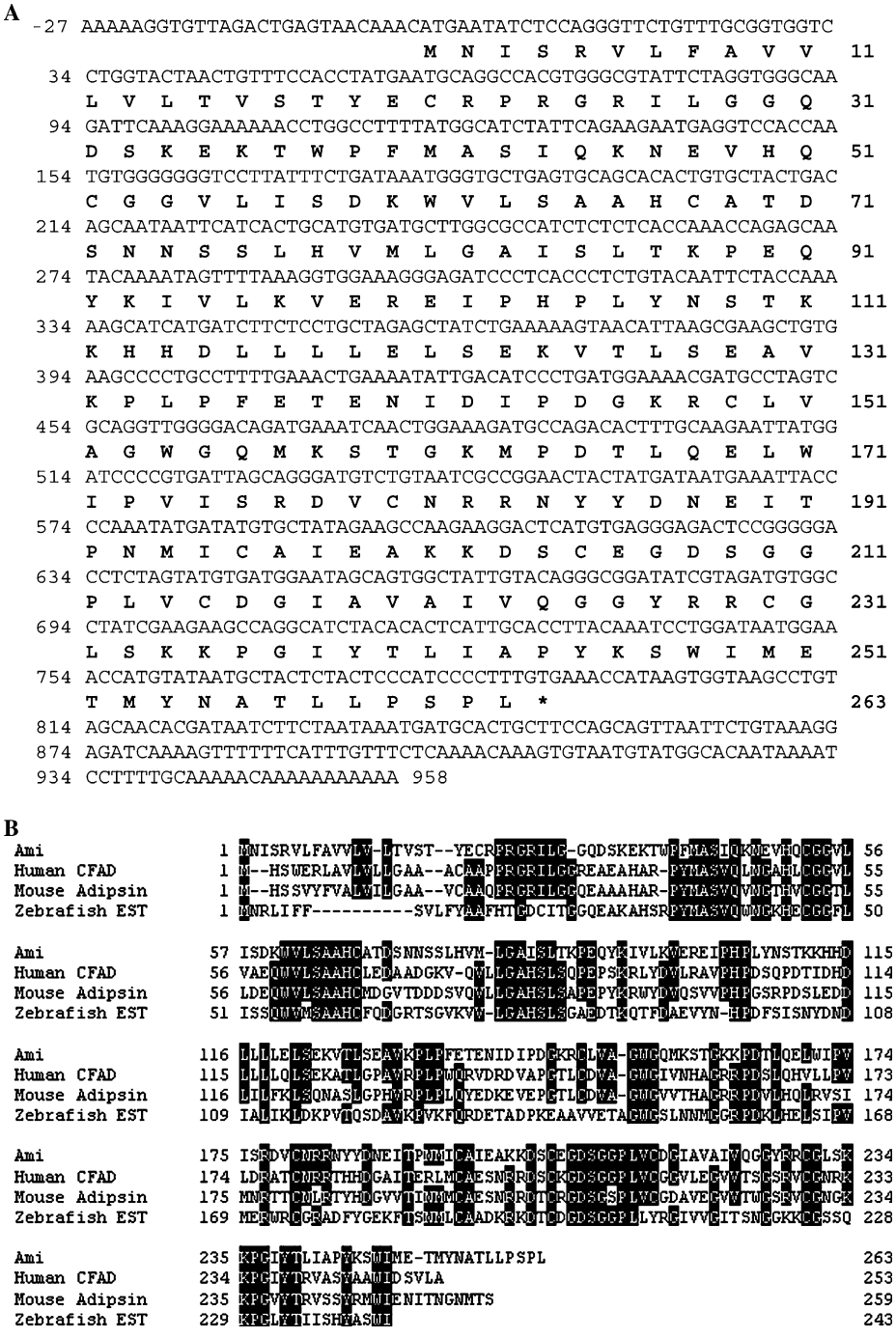

Fig. 1. Primary sequences of *Ami* cDNA and Ami protein. Nucleotide sequence of *Ami* cDNA and putative amino acid sequence of Ami protein (A). The *Ami* cDNA sequence is depicted in the upper row and deduced amino acid sequence in the lower row. Amino acid residues are presented as bold characters under the first nucleotides of the corresponding codons. The position of the stop codon is depicted by *. The numbers on the left side and the bottom refer to the cDNA sequence (+1 corresponding to the A of the first ATG codon) and the numbers on the right side refer to the amino acid sequence. Comparison of amino acid sequences homologous to Ami among species (B). Deduced amino acid sequences of Ami, human Complement Factor D (CFAD, GenBank Accession No. BC057807), mouse adipsin (GenBank Accession No. NM013459), and a Zebrafish EST with sequence similarity to human CFAD (GenBank Accession No. CF997239) are aligned. Black boxes indicate the amino acid residues that are conserved in more than three of the four sequences. Numbers refer to the amino acid sequences. Gaps are introduced to maximize the sequence similarity.

Fig. 1B. Ami demonstrated 47%, 42%, and 42% identities to human CFAD, mouse adipsin, and a Zebrafish EST similar to human CFAD (GenBank Accession No. CF997239), respectively, at the amino acid level (Fig. 1B). Since *Ami* is the most similar gene to *CFAD*/*Adipsin* in the *Xenopus laevis* EST database (NCBI), and *CFAD* is the most similar gene to *Ami* in the human genome database (NCBI), it is possible to consider *Ami* as *Xenopus* orthologue of *CFAD*/*Adipsin*. (Indeed, MGC program registered a *Xenopus tropicalis* homologous sequence