Zebrafish elav/HuC homologue as a very early neuronal marker

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Abstract

Drosophila ELAV, a neuron-specific RNA binding protein, is expressed in all neurons right after their birth. This specific pattern of expression has led to its use as a pan-neuronal marker. At least three members of the elav family, HuD, HuC/ple21 and Hel-N1, have been reported to be neuron-specific in vertebrates, although it is unknown which member of this family is expressed at the time of early neuronal determination. We have isolated a zebrafish elav/HuC homologue (zHuC) which has 89% homology to human HuC protein. It is first expressed in the neuronal precursor cells in the neural plate immediately after gastrulation, and then high expression levels persist in most regions of the nervous system. HuC, like elav in Drosophila, may be one of the earliest neuronal markers in zebrafish.

Keywords: Zebrafish elav/HuC homologue; Hu proteins; Neuron-specific RNA binding proteins; Neuronal marker; Neuronal determination; Early neurogenesis

The Drosophila elav gene was first identified in an embryonic lethal abnormal visual system phenotype with defects in the development of young neurons, suggesting that it might play an important role in the development and maintenance of the nervous system. ELAV protein has three copies of an RNA binding domain called the RNA recognition motif (RRM) and is believed to be involved in the post-transcriptional regulation of neuronal RNA. Its expression is initiated early in embryogenesis only in cells determined to become neurons in both the central and the peripheral nervous systems and is found in all stages of neuronal development. This specific, ubiquitous, and continuous pattern of elav expression has made it a useful molecular marker of neuronal differentiation [1].

In vertebrates, human elav homologues were first isolated as Hu antigens reacting with autoantibodies in patients with paraneoplastic neurologic syndrome and small cell lung cancer [2,3]. Recently, it has been reported that an anti-Hu antibody stains cells that appear in avian neurogenic populations at neuronal birthday [4,5]. However, there are at least three neuronal members of the elav family in vertebrates, HuD, HuC/ple21, and Hel-N1, all of which react with the anti-Hu antibody. It is unknown which member of this vertebrate family is expressed at the time of early neuronal determination as elav in Drosophila.

In the present study, we have isolated a zebrafish elav/HuC homologue (zHuC) 1, and examined its temporal and spatial expression during early development by whole-mount in situ hybridization. A zebrafish embryo cDNA library [6] was screened with a probe which was derived from a reverse transcription-polymerase chain reaction (RT-PCR) fragment based on the known partial cDNA sequence, starting at the EAEEA stretch in the amino acid sequence [7]. In total, 28 positive clones were isolated from $8 \times 10^5$ recombinant clones. The full coding sequence was determined after DNA sequencing of several positive clones using the dye deoxy terminator DNA sequencing system (373A, Applied Biosystems). For

1 The zHuC probe (GenBank, U62018) will be distributed to interested parties upon request.

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whole-mount in situ hybridization [8], formaldehyde-fixed embryos were treated with protease and refixed with 4% paraformaldehyde. After hybridization with digoxigenin (DIG)-labeled antisense RNA probes, samples were washed under high stringency condition and incubated in alkaline phosphatase-coupled anti-DIG antibody. After extensive washing and staining, embryos were cleared with benzyl benzoate and photographed with Nomarski optics.

The amino acid sequence deduced from the nucleotide sequences of the isolated clones showed a high level of sequence homology to other known elav-related genes [7], especially to the HuC protein (Fig. 1). The protein structure contained conserved domain arrangements, a short N-terminal domain followed by two consecutive RRMs, a linker domain, and a C-terminal RR. We also identified a clone with an in-frame deletion in the linker domain and named it zHuCmex in accordance with human HuDmex. The deletion site and size (13 amino acids deletion) were the same as those in the human HuD gene, probably due to alternative splicing [2,9]. Although the functional significance of these splicing products is unknown, the highly conserved deletion pattern within these genes suggests some role in their function. The Hu proteins share the property of binding to the 3′ untranslated regions of mRNAs, such as c-myc, c-fos, GM-CSF, and the helix-loop-helix transcription factor Id [9]. These results imply a possible function of Hu proteins in promoting neuronal differentiation by suppressing neuroblast cell proliferation.

By whole-mount in situ hybridization, HuC mRNA expression was first detected in scattered cells in the flat neural plate immediately after gastrulation (Fig. 2A). Although the time of neural cell fate determination has been described by various cell lineage experiments, it is still unclear due to a lack of available molecular markers [10–12]. Our data suggest that HuC is a useful marker for early neuronal cell fate determination in zebrafish, and its unique pattern of expression implies a role in cell fate determination in relation to that of the Notch gene (manuscript in preparation), a neurogenic gene which mediates lateral inhibition [13].

Shortly after their appearance in the neural plate, some of these scattered cells became aggregated into clusters of trigeminal ganglion neurons (Fig. 2B,C) and some others were later localized in a segmental array in the spinal cord (Fig. 2F). Positive signals were also detected in the brain region (Fig. 2A,B), although they were unidentifiable by any other known neuronal marker at this early stage. As the neural tube was formed, HuC-positive cells were localized in the ventromedial region of the spinal cord, while others were located in the dorsolateral region (Fig. 2D). Subsets of these HuC-positive cells in the spinal cord could be identified by indirect comparison with the expression pattern of a known neuronal marker, Islet-1 [6,14], and with that of HNK-1 antibody staining which was
detected in the clusters of trigeminal ganglion neurons a few hours after HuC expression [15,16]. As neurogenesis proceeds, the number of cells expressing HuC increased in the nervous system, probably due to an increase in newly emerged postmitotic neurons. High levels of HuC expression were observed in most regions of the nervous system of 32 h old embryos, including all cranial ganglia (data not shown) and firstly developing retinal ganglion cells [17]. Such high level of HuC expression persisted in adult nervous system as with human HuC and Drosophila elav gene.

The present results indicate that HuC is one of the earliest neuronal markers for early identification of neuronal determination in zebrafish, and that it may also exist in...
other vertebrates. Since the elav/Hu genes are well conserved from Drosophila to man, it is likely that they are involved in a fundamental mechanism underlying neuronal cell fate determination.


